Dear Colleagues,

It is with great pleasure we welcome you to the Fifth International Congress of Hair Research.

The purpose of this conference is to bridge the basic scientist and clinician in their pursuit to help patients with hair diseases. This exchange of ideas usually has a major impact on the field of trichologic medicine and helps determine directions of research. It stimulates interactions and development in the field. Basic science and clinical topics will be showcased in the form of workshops, plenary lectures, satellite sessions and poster presentations.

We have brought together a dynamic group of Invited Speakers for the pre-conference workshops and to provide invited lectures throughout the program. We would like to thank the Scientific Advisory Committee for their work in reviewing and selecting the abstract presentations for this program. And we are especially grateful to our corporate sponsors, without whom this congress would not have been possible.

Vancouver is such a spectacular city and we hope that you will take full advantage of the beautiful sites, wonderful cuisine and diverse culture. The downtown core is surrounded by a natural harbour and majestic mountains and offers site-seeing, beaches, shipping, restaurants and Stanley Park – all within walking distance. We encourage you to set out on foot and enjoy the scenery.

Jerry Shapiro, MD     George Cotsarelis, MD
Conference Chair     NAHRS President
Scientific Advisory Board

**Conference Chair**
Jerry Shapiro, MD  
University of British Columbia, Vancouver, BC, Canada

**NAHRS President**
George Cotsarelis, MD  
University of Pennsylvania, Philadelphia, PA, USA

**North America**
Wilma Bergfeld, MD, PhD  
The Cleveland Clinic Foundation, Cleveland, OH, USA
Vladimir Botchkarev, MD, PhD  
Boston University School of Medicine, Boston, MA, USA
Angela Christiano, PhD  
Columbia University, New York, NY, USA
Cheng Ming Chuong, MD, PhD  
University of Southern California, Los Angeles, CA, USA
Andrzej Dlugosz, MD  
University of Michigan Medical School, Ann Arbor, MI, USA
Maria Hordinsky, MD  
University of Minnesota, Minneapolis, MN, USA
Kevin McElwee, PhD  
University of British Columbia, Vancouver, BC, Canada
Sarah Millar, PhD  
University of Pennsylvania, Philadelphia, PA, USA
Elise Olsen, MD  
Duke University, Durham, NC, USA
Anthony Oro, MD, PhD  
Stanford University, Stanford, CA, USA
Vera Price, MD  
University of California, San Francisco, CA, USA
Janet Roberts, MD  
NAHRS, Portland, OR, USA
Kurt Stenn, MD  
Aderans Research Institute, Philadelphia, PA, USA
Ken Washenik, MD, PhD  
Bosley Medical Group, Beverly Hills, CA, USA
David Whiting, MD  
Dallas Associated Dermatologists, Dallas, TX, USA

**Europe**
Bruno Bernard, PhD  
L’Oreal, Clichy, France
Ulrike Blume-Peytavi, MD  
Humboldt University of Berlin, Berlin, Germany
Francisco Camacho-Martinez, MD  
Avenida Republica Argentina, Seville, Spain
Rolf Hoffman, MD  
Freiburg University, Freiburg, Germany
Andrew Messenger, MD  
Royal Hallamshire Hospital, Sheffield, United Kingdom
Ralf Paus, MD  
University Hospital Eppendorf, University of Hamburg, Hamburg, Germany
Valerie Randall, PhD  
University of Bradford, West Yorkshire, United Kingdom
Antonella Tosti, MD  
University of Bologna, Bologna, Italy
Ralph Trüb, MD  
University Hospital Zurich, Zurich, Switzerland
Dominique van Neste, MD  
Skinterface, Tournai, Belgium

**Asia**
Seiji Arase, MD  
School of Medicine, University of Tokushima, Tokushima, Japan
Satoshi Itami, MD, PhD  
Osaka University Graduate School of Medicine, Osaka, Japan
Kensei Katsuoka, MD  
Kitasato University School of Medicine, Kanagawa, Japan
Byung In Ro, MD, PhD  
Myongji Hospital, Kwandong University College of Medicine, Koyang, Kyunggi, Korea
Hideoki Ogawa, MD, PhD  
Juntendo University, School of Medicine, Tokyo Japan
Ryoji Tsuboi, MD, PhD  
Tokyo Medical University, Tokyo, Japan

**Australia**
Philip Hynd, PhD  
University of Adelaide, Adelaide, SA, Australia
Rodney Sinclair, MD  
University of Melbourne, Melbourne, Vic, Australia
General Congress Information

Registration Desk Hours (BC Foyer):
Name badges must be worn at all times during the Scientific Program Sessions and Social Events. The registration desk staff would be pleased to answer any questions about Vancouver or the Congress.

Tuesday, June 12 16:00 – 20:00
Wednesday, June 13 07:30 – 20:00
Thursday, June 14 07:00 – 17:00
Friday, June 15 07:00 – 17:00
Saturday, June 16 07:30 – 17:00

Speaker Services Centre (Tweedsmuir Room)
Speakers are asked to bring a copy of the presentations on a CD or memory stick in PC format to the Speaker Services Centre 24 hours prior to your presentation. Computers will be available for revisions.

Exhibit Area Hours (British Ballroom)

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thursday, June 14</td>
<td>10:00 – 17:00</td>
</tr>
<tr>
<td>Friday, June 15</td>
<td>10:30 – 17:00</td>
</tr>
<tr>
<td>Saturday, June 16</td>
<td>09:30 – 12:30</td>
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</table>

Accreditation
The North American Hair Research Society International Congress of Hair Research is recognized by the American Academy of Dermatology for 26 Hours of AAD category 1 CME credit and may be used toward the American Academy of Dermatology’s Continuing Medical Education Award.

Program Session Codes
WS – Workshop  O – Oral Abstract
S – Invited Speaker  P – Poster Abstract

Session 4B: Animal and Human Pathology
Thursday, June 14th, 15:30 to 17:00
For those attending this session, the session speakers have arranged sample slides for viewing. Microscopes will be available beginning on Wednesday morning until the end of the day. Please ask the Registration Desk staff for the location of the slide viewing.

Poster Presentations
Posters are located in the British Ballrom and will be available for viewing until Saturday noon. A poster session will be held on Friday, June 15th from 15:00 to 17:30. Presenters will stand by their posters to answer questions during the following times:
- 15:30 – 16:30 Session 1: even numbered posters
- 16:30 – 17:30 Session 2: odd numbered posters
Poster presenters are reminded to remove their posters from the British Ballroom by 13:00 hrs on Saturday, June 16th. Congress Organizers cannot take responsibility for posters left in the room after this time.

Congress Language
The official language of the Congress is English.

Message and Job Posting Board
Will be available by the registration desk.

Social Program

Welcome Reception
All Delegates and Registered Accompanying Persons are invited to attend.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Venue</th>
<th>Dress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wednesday, June 13th</td>
<td>18:30 – 21:00</td>
<td>The Roof, Fairmont Hotel Vancouver</td>
<td>Business Casual</td>
</tr>
</tbody>
</table>

Join us for drinks and hors d’oeuvres. Mingle with friends - old and new - a great way to begin the 5th International Congress of Hair Research.

Evening Social Event: Reception-style Dinner at the Vancouver Aquarium and Science Centre
All Delegates and Registered Accompanying Persons are invited to attend.

<table>
<thead>
<tr>
<th>Date</th>
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<th>Venue</th>
<th>Dress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friday, June 15th</td>
<td>18:30 – 22:00</td>
<td>Shuttle transfers to the Vancouver Aquarium</td>
<td>Business Casual</td>
</tr>
</tbody>
</table>

Shuttle Service: Shuttle transfers to the Vancouver Aquarium will be available. Please meet in the hotel lobby for transfer service, beginning at 18:00.
# Program

**Tuesday, June 12, 2007**

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speakers</th>
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<tbody>
<tr>
<td>16:00 – 20:00</td>
<td>Registration Open</td>
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<tr>
<td><strong>BC Foyer</strong></td>
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**Wednesday, June 13, 2007**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session #</th>
<th>Topic</th>
<th>Speakers</th>
</tr>
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<tbody>
<tr>
<td>07:30 – 20:00</td>
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<td>Registration Open</td>
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<tr>
<td><strong>BC Foyer</strong></td>
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<tr>
<td>08:00 – 12:00</td>
<td></td>
<td>AM Pre-Conference Workshops</td>
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**Room: Vancouver Island**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session #</th>
<th>Topic</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00 – 08:20</td>
<td>WS-1-A</td>
<td>Etiology of Pattern Hair Loss</td>
<td>Andrew Messenger</td>
</tr>
<tr>
<td>08:20 – 08:40</td>
<td>WS-1-B</td>
<td>Clinical Presentation, Workup PHL</td>
<td>Elise Olsen</td>
</tr>
<tr>
<td>08:40 – 08:55</td>
<td>WS-1-C</td>
<td>Management of Male Pattern Hair Loss</td>
<td>Dominique van Neste</td>
</tr>
<tr>
<td>08:55 – 09:05</td>
<td>WS-1-D</td>
<td>Pathology of PHL</td>
<td>Wilma Bergfeld</td>
</tr>
<tr>
<td>09:05 – 09:25</td>
<td>WS-1-E</td>
<td>Pattern Hair Loss – Medical Therapy</td>
<td>Jerry Shapiro</td>
</tr>
<tr>
<td>09:25 – 09:45</td>
<td>WS-1-F</td>
<td>Surgical Management of Pattern Hair Loss</td>
<td>Ken Washenik</td>
</tr>
<tr>
<td>09:45 – 10:00</td>
<td>WS-1-G</td>
<td>In Search Of Therapies For Pattern Hair Loss: Defining The Challenges And Searching For Solutions</td>
<td>Satoshi Itami</td>
</tr>
<tr>
<td>10:00 – 10:15</td>
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<td>Questions</td>
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<tr>
<td>10:15 – 10:40</td>
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<td>Refreshment Break</td>
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<tr>
<td>10:40 – 11:20</td>
<td></td>
<td>Patient Viewing</td>
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<tr>
<td>11:20 – 12:00</td>
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<td>Patient Discussion</td>
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**Room: Waddington**

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<tr>
<th>Time</th>
<th>Session #</th>
<th>Topic</th>
<th>Speakers</th>
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</thead>
<tbody>
<tr>
<td>08:00 – 08:20</td>
<td>WS-2-A</td>
<td>Classification and Clinical Presentation</td>
<td>Ralph Trüeb</td>
</tr>
<tr>
<td>08:20 – 08:30</td>
<td>WS-2-B</td>
<td>Histopathology of Cicatricial Alopecia</td>
<td>David Whiting</td>
</tr>
<tr>
<td>08:30 – 08:45</td>
<td>WS-2-C</td>
<td>Etiology of Cicatricial Alopecia: Background and Hypotheses to Test</td>
<td>Kurt Stenn</td>
</tr>
<tr>
<td>08:45 – 08:55</td>
<td>WS-2-D</td>
<td>CD200 Attenuates Hair Follicle-specific Inflammation in Mice</td>
<td>Michael Rosenblum</td>
</tr>
<tr>
<td>08:55 – 09:15</td>
<td>WS-2-E</td>
<td>PPAR Gamma Deletion in Stem Cells of the Hair Follicle Causes Scarring Alopecia</td>
<td>Pratima Karnik</td>
</tr>
<tr>
<td>09:15 – 09:30</td>
<td>WS-2-F</td>
<td>CCCA Update</td>
<td>Elise Olsen</td>
</tr>
<tr>
<td>09:30 – 09:40</td>
<td>WS-2-G</td>
<td>Secrets of Cicatricial Alopecias Revealed by Mouse Models</td>
<td>John Sundberg</td>
</tr>
<tr>
<td>09:40 – 09:55</td>
<td>WS-2-H</td>
<td>Cicatricial Alopecia: A Practical Diagnosis and Therapeutic Approach</td>
<td>Pascal Reygagne</td>
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<tr>
<td>10:10 – 10:15</td>
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<td>Questions</td>
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<td>10:15 – 10:40</td>
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<td>Refreshment Break</td>
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<td>11:20 – 12:00</td>
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<td>Patient Discussion</td>
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<td>12:00 – 13:00</td>
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<td>Lunch Break (delegates on their own)</td>
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### Program

**Wednesday, June 13, 2007**

<table>
<thead>
<tr>
<th>Time</th>
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<tr>
<td>13:00 – 16:30</td>
<td>PM Pre Conference Workshops</td>
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<tr>
<td><strong>Room:</strong></td>
<td><strong>Vancouver Island</strong></td>
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<tr>
<td><strong>Alopecia Areata Pre-Conference Workshop 3</strong></td>
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<tr>
<td>13:00 – 13:20</td>
<td>WS-3-A</td>
<td>Genetics</td>
<td>Angela Christiano</td>
</tr>
<tr>
<td>13:35 – 13:50</td>
<td>WS-3-C</td>
<td>Histopathology of Alopecia Areata</td>
<td>David Whiting</td>
</tr>
<tr>
<td>13:50 – 14:10</td>
<td>WS-3-D</td>
<td>Clinical Features</td>
<td>Maria Hordinsky</td>
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<tr>
<td>14:10 – 14:30</td>
<td>WS-3-E</td>
<td>Alopecia Areata – Topical Immunotherapy</td>
<td>Jerry Shapiro</td>
</tr>
<tr>
<td>14:30 – 15:00</td>
<td>WS-3-F</td>
<td>Biologics, Calcineurin Inhibitors and What’s Ahead in Alopecia Areata</td>
<td>Vera Price</td>
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<tr>
<td>15:00 – 15:10</td>
<td></td>
<td>Questions</td>
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<tr>
<td>15:10 – 15:25</td>
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<td>Refreshment Break</td>
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<td>Patient Viewing</td>
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<td>16:00 – 16:30</td>
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<td>Patient Discussion</td>
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<tr>
<td><strong>Room:</strong></td>
<td><strong>Waddington</strong></td>
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<tr>
<td><strong>Hirsutism Pre-Conference Workshop 4</strong></td>
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<tr>
<td>13:00 – 13:20</td>
<td>WS-4-A</td>
<td>Aetiology of Hirsutism</td>
<td>Valerie Randall</td>
</tr>
<tr>
<td>13:20 – 13:40</td>
<td>WS-4-B</td>
<td>Presentation and Evaluation of Hirsutism</td>
<td>Ulrike Blume-Peytavi</td>
</tr>
<tr>
<td>13:40 – 13:50</td>
<td>WS-4-C</td>
<td>Hirsutism in Children</td>
<td>Danielle Marcoux</td>
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<tr>
<td>13:50 – 14:20</td>
<td>WS-4-D</td>
<td>Treatment of the Hirsute Patient</td>
<td>Wilma Bergfeld</td>
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<tr>
<td>14:20 – 14:55</td>
<td>WS-4-E</td>
<td>Lasers and the Future</td>
<td>Rox Anderson</td>
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<tr>
<td>14:55 – 15:05</td>
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<td>Questions</td>
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<td>15:05 – 15:25</td>
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<td>16:00 – 16:30</td>
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<td>Patient Discussion</td>
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<tr>
<td>16:30 – 18:00</td>
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<td>Satellite Symposium - Unwanted Hair Growth</td>
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<tr>
<td><strong>Room:</strong></td>
<td><strong>Pacific Ballroom</strong></td>
<td>Treatment Options and Impact on Quality of Life</td>
<td>Shire, SkinMedica, Barrier Therapeutics</td>
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<tr>
<td>18:30 – 21:00</td>
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<td>Opening Reception</td>
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**Thursday, June 14, 2007**

<table>
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<tr>
<th>Time</th>
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<tr>
<td>07:00 – 17:00</td>
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<td>Registration Open</td>
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<td><strong>BC Foyer</strong></td>
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<tr>
<td><strong>Room:</strong></td>
<td><strong>Pacific Ballroom</strong></td>
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<tr>
<td>Opening Addresses</td>
<td></td>
<td></td>
<td>Jerry Shapiro</td>
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<tr>
<td>07:45 – 07:50</td>
<td></td>
<td>Opening Address from Conference Chair</td>
<td>George Cotsarelis</td>
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<tr>
<td>07:50 – 07:55</td>
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<td>Opening Address from NAHRS President</td>
<td>Rolf Hoffman</td>
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<tr>
<td>07:55 – 08:00</td>
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<td>Organization Address – EHRS</td>
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<table>
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<tr>
<th>Time</th>
<th>Session #</th>
<th>Topic</th>
<th>Speakers</th>
</tr>
</thead>
</table>
| 08:00 – 10:00 | **Pacifi c Ballroom** | **Session 1**  
Stem Cells  
Co-chairs:  
George Cotsarelis, Colin Jahoda, Takashi Matsuzaki, | Co-chairs:  
George Cotsarelis, Colin Jahoda, Takashi Matsuzaki, |
| 08:00 – 08:50 | S-1-A     | Cancer-Initiating Cells: From Leukemia to Solid Tumors                | John Dick                                                              |
| 08:50 – 09:00 | Questions |                                                                        |                                                                         |
| 09:00 – 09:15 | S-1-B     | Hair Follicle Stem Cells – Epithelial                                 | George Cotsarelis                                                       |
| 09:15 – 09:20 | Questions |                                                                        |                                                                         |
| 09:20 – 09:30 | O-01-1    | Stem Cells of Human Hair Follicles Can Differentiate Into Neurons: Region-Specific Multipotency of Human Hair Follicle Stem Cells | Yasuyuki Amoh                                                          |
| 09:30 – 09:40 | O-01-2    | Adult Stem Cell Compartment Changes in Androgenetic Alopecia Demonstrate Maintenance of Progenitor Stem Cells With Loss of Descendant CD200 High A6 Integrin High Expressing Cells | Luis Garza                                                             |
| 09:40 – 09:50 | O-01-3    | Adult Hair Follicle Dermal Papillae Induce Hair and Skin Differentiation From Adult Corneal Epithelium | James Waters                                                            |
| 09:50 – 10:00 | O-01-4    | Bone Morphogenetic Protein Signaling is Required for Hair Induction by Dermal Papilla Cells | Michael Rendl                                                          |
| 10:00 – 17:00 | **British Ballroom** | **Exhibition and Poster Viewing**                                       |                                                                         |
| 10:30 – 12:15 | **Pacifi c Ballroom** | **Session 2**  
Mesenchymal Stem Cells  
Co-chairs:  
Andrew Messenger, Manabu Ohyama | Co-chairs:  
Andrew Messenger, Manabu Ohyama |
| 10:30 – 10:35 | Introduction |                                                                        |                                                                         |
| 10:35 – 11:25 | S-2-A     | Skin-derived Precursors (SKPs) and Induction of Hair Follicle Morphogenesis | Freda Miller                                                            |
| 11:25 – 11:50 | S-2-B     | Manipulating Gene Expression in the Dermal Papilla of the Mouse in Vivo | Bruce Morgan                                                            |
| 11:50 – 12:15 | S-2-C     | Mesenchymal-Epithelial Interactions Needed for Tissue Engineering of Hair Follicles | Colin Jahoda                                                            |
| 12:15 – 13:45 | **Columbia Ballroom** | **Satellite Symposium – Ethnic Hair**                                    |                                                                         |
| 13:45 – 15:00 | **Pacifi c Ballroom** | **Session 3**  
Tissue Engineering  
Co-chairs:  
Satoshi Itami, Valerie Randall, Michael Philpott | Co-chairs:  
Satoshi Itami, Valerie Randall, Michael Philpott |
| 13:45 – 14:00 | S-3       | Tissue Engineering of Hair follicles                                   | Kurt Stenn                                                              |
| 14:00 – 14:10 | O-03-1    | Regeneration of Human-Mouse Chimeric Follicles in a Hybrid Patch Assay | Ying Zheng                                                              |
| 14:10 – 14:20 | O-03-2    | Methods of Follicular Cell Implantation for Hair Multiplication         | Jeff Teumer                                                             |
| 14:20 – 14:30 | O-03-3    | Expression of TGF Beta2 in Cultured Human Dermal Papilla Cells and Its Ability of Induction of Tissue Engineered Hair Follicles | Keita Inoue                                                             |
### Program

**Thursday, June 14, 2007 – continued**

<table>
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<tr>
<th>Time</th>
<th>Session #</th>
<th>Topic</th>
<th>Speakers</th>
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<tbody>
<tr>
<td>14:30 – 14:40</td>
<td>O-03-4</td>
<td>In Vitro Generation of Human Hair Follicle Bud Oriented Cellular Mass Composed of Dermal Papilla Cells and Keratinocytes</td>
<td>Shigeyoshi Fuziwara</td>
</tr>
<tr>
<td>14:40 – 14:50</td>
<td>O-03-5</td>
<td>The Hair-Inducing Clonal Cell Lines From Dermal Papilla and Dermal Sheath Cells of Mouse Vibrissa Follicles</td>
<td>Aki Osada</td>
</tr>
<tr>
<td>14:50 – 15:00</td>
<td>O-03-6</td>
<td>Large-Scale Production of Dermal Papilla Microtissues Via Facilitated Self-Assembling: Implications For Hair Follicle Engineering and Dermal Papilla Physiology</td>
<td>Sung-Jan Lin</td>
</tr>
<tr>
<td>15:00 – 15:30</td>
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<td>Refreshment Break</td>
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<tr>
<td><strong>Room:</strong></td>
<td><strong>British Ballroom</strong></td>
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<tr>
<td>15:30 – 17:00</td>
<td>Session 4A</td>
<td>Hair Surgery</td>
<td>Co-chairs: Walter Unger, Ken Washenik, Arthur Tykocinski</td>
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<tbody>
<tr>
<td>15:30 – 15:45</td>
<td>S-4A-a</td>
<td>Update on Concepts and Techniques in Hair Transplantation 2007</td>
<td>Walter Unger</td>
</tr>
<tr>
<td>15:45 – 16:00</td>
<td>S-4A-b</td>
<td>Evidence Based Hair Restoration</td>
<td>Andreas Finner</td>
</tr>
<tr>
<td>16:00 – 16:15</td>
<td>S-4A-c</td>
<td>Racial Differences in Hair Transplantation</td>
<td>Valerie Callendar</td>
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<tr>
<td>16:15 – 16:30</td>
<td>S-4A-d</td>
<td>Hair Restoration Surgery in Unusual Cases: Cicatricial Alopecia, Congenital Alopecia and Other Alopecias</td>
<td>Nilofer Farjo</td>
</tr>
<tr>
<td>16:30 – 16:40</td>
<td>O-4A-1</td>
<td>Re-evaluation of Natural Hairline Patterns and Recession Patterns of the Frontal and Midscalp Zones in Men</td>
<td>William Parsley</td>
</tr>
<tr>
<td>16:50 – 17:00</td>
<td>O-4A-3</td>
<td>Hair Transplant in Asians</td>
<td>Eileen Tan</td>
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**Room:** Waddington

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 4B</th>
<th>Pathology: Animal and Human Pathology</th>
<th>Co-chairs: Magnalena Martinka, John Sundberg, David Whiting</th>
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</thead>
<tbody>
<tr>
<td>15:30 – 17:00</td>
<td>S-4B</td>
<td>Histopathology of Selected Mouse Models for Human Hair Diseases</td>
<td>David Whiting, Magnalena Martinka, John Sundberg</td>
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</table>

**Room:** Vancouver Island

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 4C</th>
<th>Congenital Hair Loss / Ectodermal Dysplasias</th>
<th>Co-chairs: Angela Christiano, Abraham Zlotogorski</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:30 – 16:30</td>
<td>S-4C</td>
<td>Review of Ectodermal Dysplasias</td>
<td>Angela Christiano</td>
</tr>
<tr>
<td>16:30 – 16:40</td>
<td>O-4C-1</td>
<td>Autosomal Dominant and Autosomal Recessive Monilethrix – Report of 28 Families</td>
<td>Abraham Zlotogorski</td>
</tr>
<tr>
<td>16:40 – 16:50</td>
<td>O-4C-2</td>
<td>Atrichia With Papular Lesions at Young Age May Be Misdiagnosed as Patchy Alopecia Areata</td>
<td>Liran Horev</td>
</tr>
<tr>
<td>16:50 – 17:00</td>
<td>O-4C-3</td>
<td>Increased Expression of EctodysplasinA1 and Ectodysplasin Receptor Coincides with the Formation of Primary Wool Follicles in Sheep</td>
<td>Hayley McGrice</td>
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**Room:** Garibaldi

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<tr>
<td>17:00 – 17:30</td>
<td></td>
<td>EHRS Board Meeting (invitation only)</td>
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<tr>
<td>17:30 – 18:00</td>
<td>EHRS</td>
<td>EHRS General Meeting (EHRS Members)</td>
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<td>Garibaldi</td>
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### Program

**Friday, June 15, 2007**

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<tr>
<th>Time</th>
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<th>Topic</th>
<th>Speakers</th>
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<tbody>
<tr>
<td>06:45 – 07:45</td>
<td></td>
<td>Satellite Closed Session (by Invitation Only) – Hair Science: From Bench-top to Bottle</td>
<td></td>
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<tr>
<td>07:00 – 17:00</td>
<td></td>
<td>Registration Open</td>
<td></td>
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<tr>
<td>07:50 – 07:55</td>
<td></td>
<td>Organization Address – Australia</td>
<td>Rodney Sinclair</td>
</tr>
<tr>
<td>07:55 – 08:00</td>
<td></td>
<td>Organization Address – Korea</td>
<td>Byung In Ro</td>
</tr>
<tr>
<td>08:00 – 10:30</td>
<td></td>
<td><strong>Session 5</strong> Morphogenesis / Follicular Cycling</td>
<td>Co-chairs: Cheng Ming Chuong, Phillip Hynd, Ralf Paus</td>
</tr>
<tr>
<td>08:00 – 08:50</td>
<td>S-5-A</td>
<td>Ebling Lecture: Wnt and Notch Signaling Pathways in Development and Cancer of the Gut</td>
<td>Hans Clevers</td>
</tr>
<tr>
<td>08:50 – 09:20</td>
<td>S-5-B</td>
<td>New Insights into Telogen</td>
<td>Cheng Ming Chuong</td>
</tr>
<tr>
<td>09:20 – 09:50</td>
<td>S-5-C</td>
<td>Wnt Signaling in the Control of Hair Follicle Development</td>
<td>Sarah Millar</td>
</tr>
<tr>
<td>09:50 – 10:00</td>
<td>O-05-1</td>
<td>Wnt-Dependent De Novo Hair Follicle Regeneration in Adult Mouse Skin Following Wounding</td>
<td>Mayumi Ito</td>
</tr>
<tr>
<td>10:00 – 10:10</td>
<td>O-05-2</td>
<td>P-Cadherin Is a p63 Target Gene With a Critical Role in the Developing Limb Bud and Hair Follicle.</td>
<td>Angela Christiano</td>
</tr>
<tr>
<td>10:10 – 10:20</td>
<td>O-05-3</td>
<td>The Wnt Inhibitor, Dickkopf 4, Is Induced By Canonical Wnt Signaling During Ectodermal Appendage Morphogenesis</td>
<td>Hisham Bazzi</td>
</tr>
<tr>
<td>10:20 – 10:30</td>
<td>O-05-4</td>
<td>Molecular Signature of the Follicular and Glandular Types of Epidermal Differentiation: Evidence That BMP Signaling Suppresses Trans-Differentiation of the Foot Pad Epidermis Towards Folliculogenesis</td>
<td>Vladimir Botchkarev</td>
</tr>
<tr>
<td>10:30 – 17:30</td>
<td></td>
<td><strong>Exhibition and Poster Viewing</strong></td>
<td></td>
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<tr>
<td>11:00 – 12:00</td>
<td></td>
<td>Session 6 Follicular Growth Controls</td>
<td>Co-chairs: Colin Jahoda, Sarah Millar, Hideoki Ogawa</td>
</tr>
<tr>
<td>11:00 – 11:30</td>
<td>S-6-A</td>
<td>Controlling Hair Follicle Morphogenesis Through Polyubiquitination</td>
<td>Anthony Oro</td>
</tr>
<tr>
<td>11:30 – 12:00</td>
<td>S-6-B</td>
<td>Hedgehog Functions in the Pilosebaceous Unit</td>
<td>Andrzej Dlugosz</td>
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<tr>
<td>12:00 – 13:30</td>
<td></td>
<td>Lunch Break – Exhibit and Poster Viewing</td>
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<tr>
<td>13:30 – 15:05</td>
<td></td>
<td><strong>Session 7A</strong> Non-Invasive and Invasive Hair Techniques for Quantifying and Visualizing Hair Growth</td>
<td>Co-chairs: Seiji Arase, Elise Olsen, Dominique Van Neste</td>
</tr>
<tr>
<td>13:30 – 13:45</td>
<td>S-7A-a</td>
<td>Hair Metrix Update</td>
<td>Doug Canfield</td>
</tr>
<tr>
<td>13:45 – 14:00</td>
<td>S-7A-b</td>
<td>GCP-Validation of TrichoScan</td>
<td>Rolf Hoffman</td>
</tr>
<tr>
<td>14:00 – 14:15</td>
<td>S-7A-c</td>
<td>Dermoscopy of Hair (video)</td>
<td>Antonella Tosti</td>
</tr>
<tr>
<td>14:25 – 14:35</td>
<td>O-7A-2</td>
<td>Methodology for the Assessment of Efficacy in Clinical Trials of Cicatrical Alopecia</td>
<td>Elise Olsen</td>
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**Friday, June 15, 2007 – continued**

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<th>Time</th>
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<tbody>
<tr>
<td>14:35 – 14:45</td>
<td>O-7A-3</td>
<td>The Role of Scalp Dermoscopy in the Diagnosis of</td>
<td>Antonella Tosti, Matilde Iorizzo</td>
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<tr>
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<td></td>
<td>Alopecia Areata Incognita</td>
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<tr>
<td>14:45 – 14:55</td>
<td>O-7A-4</td>
<td>Visualizing Hair Follicle-Associated Lymphatics in</td>
<td>Marna Ericson</td>
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<td>Human and Murine Skin</td>
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<tr>
<td>14:55 – 15:05</td>
<td>O-7A-5</td>
<td>Comparison of Hair Growth Parameters in Pre- and</td>
<td>Thomas Dawson</td>
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<td>Post-Menopausal Women Using a Digital Macrograph</td>
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<td>Imaging System</td>
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<tr>
<td>14:15 – 14:55</td>
<td></td>
<td><strong>Room:</strong> Pacific Ballroom</td>
<td><strong>Co-chairs:</strong> Vera Price, Byung In Ro,</td>
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<tr>
<td></td>
<td></td>
<td><strong>Session 7B – Alopecia Areata</strong></td>
<td>Antonella Tosti</td>
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<tr>
<td>14:15 – 14:25</td>
<td>O-7B-1</td>
<td>Haplotype Analysis Identifies a Key Network in the</td>
<td>John Sundberg</td>
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<td></td>
<td>Pathogenesis of Alopecia Areata in Mice</td>
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<tr>
<td>14:25 – 14:35</td>
<td>O-7B-2</td>
<td>Retinoic Acid Synthesis and Degradation Enzymes and</td>
<td>Helen Everts</td>
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<tr>
<td></td>
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<td>Binding Proteins Are Altered in Alopecia Areata</td>
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<tr>
<td>14:35 – 14:45</td>
<td>O-7B-3</td>
<td>Alopecia Areata in Scotland – Results of a</td>
<td>Megan Mowbray</td>
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<td>Questionnaire Study</td>
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<tr>
<td>14:45 – 14:55</td>
<td>O-7B-4</td>
<td>Genomewide Scan For Linkage Reveals Evidence of</td>
<td>Angela Christiano</td>
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<td></td>
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<td>Several Susceptibility Loci For Alopecia Areata</td>
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<tr>
<td>13:30 – 15:00</td>
<td></td>
<td><strong>Room:</strong> Waddington</td>
<td><strong>Co-chairs:</strong> Ralf Paus, Michael Philpot</td>
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<tr>
<td>13:30 – 14:30</td>
<td>S-7C</td>
<td>Overview</td>
<td>Ralf Paus</td>
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<tr>
<td>14:30 – 14:40</td>
<td>O-7C-1</td>
<td>Odor Restores Hair Cycle Delay Caused by Immobilization Stress</td>
<td>Jiro Kishimoto</td>
</tr>
<tr>
<td>14:40 – 14:50</td>
<td>O-7C-2</td>
<td>Stress Response in a Mouse Model of Alopecia Areata</td>
<td>Xingqi Zhang</td>
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<tr>
<td>14:50 – 15:00</td>
<td>O-7C-3</td>
<td>Altered Peripheral Nerve Function in Alopecia Areata</td>
<td>Eric Cornatzer</td>
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<tr>
<td>15:00 – 17:30</td>
<td></td>
<td><strong>Poster Session</strong></td>
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<td>15:30 – 16:30</td>
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<td>Poster session (even numbered posters)</td>
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<tr>
<td>16:30 – 17:30</td>
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<td>Poster session (odd numbered posters)</td>
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<tr>
<td>18:30 – 22:00</td>
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<td><strong>Gala Event (Vancouver Aquarium &amp; Science Centre)</strong></td>
<td>transfer shuttles begin loading at 18:00</td>
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<tr>
<td>07:30 – 17:00</td>
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<td>Registration Open</td>
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<tr>
<td><strong>BC Foyer</strong></td>
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<tr>
<td>07:55 – 08:00</td>
<td></td>
<td>Organization Address – Japan</td>
<td>Seiji Arase</td>
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<tr>
<td><strong>Room:</strong></td>
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<tr>
<td><strong>Pacific Ballroom</strong></td>
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<tr>
<td>08:00 – 09:30</td>
<td></td>
<td><strong>Session 8 Hair Pigmentation</strong></td>
<td><strong>Co-chairs:</strong> Greg Barsh, Emi Nishimura,</td>
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<td></td>
<td>Desmond Tobin</td>
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<tr>
<td>08:00 – 08:50</td>
<td>S-8-A</td>
<td>Hair Pigmentation</td>
<td>Greg Barsh</td>
</tr>
<tr>
<td>08:50 – 09:20</td>
<td>S-8-B</td>
<td>Mechanisms of Melanocyte Stem Cell Maintenance and</td>
<td>Emi Nishimura</td>
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<td>Hair Graying</td>
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<tr>
<td>Time</td>
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<td>Speakers</td>
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<tr>
<td>09:20 – 09:30</td>
<td>O-08-1</td>
<td>Processing of Proopiomelanocortin in Melanocytes of the Human Hair Follicle and Epidermis – Implications For Regulation of Melanogenesis</td>
<td>Desmond Tobin</td>
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<tr>
<td>09:30 – 10:00</td>
<td>S-9</td>
<td>Molecular Mechanisms of Chemotherapy-Induced Hair Loss: Global Changes in Expression of Apoptotic and Non-Apoptotic Genes During the Response of Human Hair Follicles to Doxorubicin</td>
<td>Vladimir Botchkarev</td>
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<tr>
<td>09:30 – 12:30</td>
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<td>Exhibition and Poster Viewing</td>
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<tr>
<td>10:00 – 10:30</td>
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<td>Refreshment Break</td>
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<tr>
<td>10:30 – 11:10</td>
<td>O-10-1</td>
<td>Focal Atrichia</td>
<td>Elise Olsen</td>
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<tr>
<td>10:40 – 10:50</td>
<td>O-10-2</td>
<td>Female Pattern Hair Loss Revisited: A Pilot Study Reveals Novel Characteristics</td>
<td>Elizabeth Ross</td>
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<tr>
<td>10:50 – 11:00</td>
<td>O-10-3</td>
<td>Comparison of Senescent and Androgenetic Alopecia Using Microarray Analysis</td>
<td>Paradi Mirmirani</td>
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<tr>
<td>11:00 – 11:10</td>
<td>O-10-4</td>
<td>The Role of the Androgen Receptor Gene CAG Repeat Polymorphism and X-Chromosome Inactivation Pattern in Postmenopausal Female Pattern Hair Loss</td>
<td>Iaishi Ali</td>
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<tr>
<td>11:25 – 11:35</td>
<td>O-11-2</td>
<td>Successful Hair Regrowth With Early Treatment of DLE Cicatricial Alopecia</td>
<td>Nina Otberg, Tatyana Hamilton</td>
</tr>
<tr>
<td>11:35 – 11:45</td>
<td>O-11-3</td>
<td>A Case Series of 29 Patients With Lichen Planopilaris-The Cleveland Clinic Foundation Experience on Evaluation, Diagnosis and Treatment</td>
<td>Nathaniel Cevasco</td>
</tr>
<tr>
<td>11:45 – 11:55</td>
<td>O-11-4</td>
<td>Clinical Spectrum of Postmenopausal Frontal Fibrosing Alopecia</td>
<td>Hans Wolff</td>
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<tr>
<td>11:55 – 12:05</td>
<td>O-11-5</td>
<td>Retinoic Acid Synthesis Enzymes and Binding Proteins Are Increased in Central Centrifugal Cicatricial Alopecia</td>
<td>Helen Everts</td>
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<tr>
<td>13:30 – 15:05</td>
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<td><strong>Session 12</strong></td>
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<tr>
<td>13:30 – 14:10</td>
<td>S-12-A</td>
<td>Laser Hair Treatments</td>
<td>R. Rox Anderson</td>
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<tr>
<td>14:10 – 14:25</td>
<td>S-12-B</td>
<td>Nanoparticle-based Targeting of Skin Antigen-Presenting Cells via Hair Follicles</td>
<td>Annika Vogt</td>
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<tr>
<td>14:25 – 14:35</td>
<td>S-12-C</td>
<td>Role of Hair Follicles of Transcutaneous Drug Delivery</td>
<td>Nina Otberg</td>
</tr>
<tr>
<td>14:35 – 14:45</td>
<td>O-12-1</td>
<td>Topical Application of Antagonist to the G-protein Coupled Receptor Smoothened of the Sonic Hedgehog Signaling Pathway Inhibits Hair Growth in C3H Mice</td>
<td>Zengquan Wang</td>
</tr>
<tr>
<td>14:45 – 14:55</td>
<td>O-12-2</td>
<td>The miRNA Processing Enzyme Dicer is Required for Hair Follicle Maintenance In Adult Skin</td>
<td>Thomas Andl</td>
</tr>
<tr>
<td>14:55 – 15:05</td>
<td>O-12-3</td>
<td>The Autoimmune Regulator (AIRE) 7215C Allele Is Strongly Associated With Failure of Diphencyprone (DPCP) Treatment in Alopecia Areata (AA): Prospect of Developing a Genetic Test to Predict Therapeutic Response</td>
<td>Andrew McDonagh</td>
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<tr>
<td>15:05 – 15:30</td>
<td>Room: BC Foyer</td>
<td>Refreshment Break</td>
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<tr>
<td>15:30 – 17:00</td>
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<td><strong>Session 13A</strong></td>
<td></td>
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<tr>
<td>15:30 – 15:50</td>
<td>S-13A-a</td>
<td>Genes in Distinct Types of Murine Hair Follicles: Involvement of Bmp Signaling in the Controlling Hair of Thickness And Shape</td>
<td>Vladimir Botchkarev</td>
</tr>
<tr>
<td>15:50 – 16:00</td>
<td>S-13A-b</td>
<td>Characterization of Human Hair Shape: From Hair Bulb to Hair Fiber</td>
<td>Bruno Bernard</td>
</tr>
<tr>
<td>16:00 – 16:10</td>
<td>O-13A-1</td>
<td>Pili Annulati-Reduction of Candidate Region to 2.9 Mb By Genetic Analysis of 4 Additional Families With Pili Annulati and Expression Analysis of Genes in the Critical Region</td>
<td>Kathrin Giehl</td>
</tr>
<tr>
<td>16:10 – 16:20</td>
<td>O-13A-2</td>
<td>Dominant Mutation in the Rod Domain of Keratin 6hf Results in Hair Phenotypes Resembling Trichorhixis Nodosa in Mice</td>
<td>Jiang Chen</td>
</tr>
<tr>
<td>16:20 – 16:30</td>
<td>O-13A-3</td>
<td>Matrix to Intermediate Filament Ratio in the Cortex of Merino Wool Correlates to Curl</td>
<td>Duane Harland</td>
</tr>
<tr>
<td>16:30 – 16:40</td>
<td>O-13A-4</td>
<td>Hair Photoaging: Ultraviolet Induced Photodegradation and Restoration of Human Hair</td>
<td>Won-Soo Lee</td>
</tr>
<tr>
<td>16:40 – 16:50</td>
<td>O-13A-5</td>
<td>Characterization of Female Facial Hair: Morphology and Growth Properties of Two Novel Subtypes of Upper Lip Terminal Hairs and Responses to Vaniqa Treatment</td>
<td>John Oblong</td>
</tr>
<tr>
<td>16:50 – 17:00</td>
<td>Room: Waddington</td>
<td>Questions &amp; Answers</td>
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<tr>
<td>15:30 – 17:00</td>
<td>Room:</td>
<td><strong>Session 13B</strong> Nutrition and Hair Growth</td>
<td>Co-chairs: Wilma Bergfeld, Hugh Rushton</td>
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<tr>
<td></td>
<td>Vancouver Island</td>
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<tr>
<td>15:30 – 15:50</td>
<td>S-13B-a</td>
<td>Nutrition and Hair Growth</td>
<td>Ralph Trüeb</td>
</tr>
<tr>
<td>15:50 – 16:10</td>
<td>S-13B-b</td>
<td>Supplements, Iron and Hair Growth: The Cleveland Experience</td>
<td>Wilma Bergfeld</td>
</tr>
<tr>
<td>16:10 – 16:30</td>
<td>S-13B-c</td>
<td>Female Pattern Hair Loss and Iron – My View</td>
<td>Hugh Rushton</td>
</tr>
<tr>
<td>16:30 – 16:40</td>
<td>O-13B-1</td>
<td>A Novel Perspective on the Significance of Ferritin in Postmenopausal Hair Loss</td>
<td>Iaisha Ali</td>
</tr>
<tr>
<td>16:40 – 16:50</td>
<td>O-13B-2</td>
<td>Iron Deficiency in Female Pattern Hair Loss, Telogen Effluvium and Controls.</td>
<td>Kate Reed</td>
</tr>
<tr>
<td>16:50 – 17:00</td>
<td>O-13B-3</td>
<td>Effect of Oral Intake of Choline-Stabilized Orthosilic Acid on Hair Tensile Strength and Morphology in Women With Fine Hair</td>
<td>R. Wickett</td>
</tr>
<tr>
<td>16:10 – 16:50</td>
<td>Room:</td>
<td><strong>Session 13C</strong> Hirsutism/Endocrinology</td>
<td>Co-chairs: Amy McMichael, Satoshi Itami</td>
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<td></td>
<td>Pacific Ballroom</td>
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<tr>
<td>16:10 – 16:20</td>
<td>O-13C-1</td>
<td>Erythropoietin: a New Player in Hair Follicle Biology</td>
<td>Enikő Bodó</td>
</tr>
<tr>
<td>16:20 – 16:30</td>
<td>O-13C-2</td>
<td>Efficacy of Laser Therapy in Hirsute Iranian Women</td>
<td>Gita Faghihi</td>
</tr>
<tr>
<td>16:30 – 16:40</td>
<td>O-13C-3</td>
<td>Novel Function of TGFβ1 as a Key Pathogenic Molecule in Androgenetic Alopecia: Potentiation of Androgen Receptor Through Smad3</td>
<td>Shigeki Inui</td>
</tr>
<tr>
<td>16:40 – 16:50</td>
<td>O-13C-4</td>
<td>Hair Follicles Express Functional Hypothalamic-Pituitary-Thyroid (HPT) Axis-Related Elements</td>
<td>Enikő Bodó</td>
</tr>
<tr>
<td>17:00 – 17:30</td>
<td>Room:</td>
<td><strong>Closing Awards Ceremony</strong></td>
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<td>Pacific Ballroom</td>
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<tr>
<td>17:30 – 18:30</td>
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<td><strong>Closing Cocktails</strong></td>
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Invited Speaker Presentations

Wednesday, June 13, 2007

Workshop 1
Pattern Hair Loss (PHL) Pre Conference

WS-1-A
Etiology of Pattern Hair Loss
Speaker: Andrew Messenger
Department of Dermatology, Royal Hallamshire Hospital, Sheffield, United Kingdom

Pattern hair loss (PHL) is due to a progressive decline in the activity of scalp hair follicles. The pathology may be regarded as a final common pathway to which a number of factors contribute. Male PHL is genetically determined and androgen-dependent. The prevalence and severity increase with age suggesting that aging mechanisms are also involved in the etiology, and there is some evidence that the same pathology can occur independently of androgens (senile alopecia). Androgens are involved in the etiology of female PHL in some women, but this has been more difficult to prove and it is possible that non-androgenic mechanisms play a more prominent role than in men. Current thinking is that the predisposition to PHL in both sexes is polygenic. Three independent studies have linked male PHL to variant regions in the androgen receptor (AR) gene. However, this alone does not explain the paternal influence on PHL as the AR gene is located on the X chromosome and is inherited from the mother. Advances in our understanding of the molecular control of hair growth are also leading to progress in the pathobiology of PHL, and integrating these findings with genetic information will form a challenge for the future.

WS-1-B
Clinical Presentation, Workup FPHL
Speaker: Elise Olsen
Duke University, Durham, NC, USA

[Abstract/summary not available at the time of printing]

WS-1-C
Management of Male Pattern Hair Loss
Speaker: Dominique van Neste
Skinterface, Tournai, Belgium

Listen, look and touch are the three key actions that characterize the first encounter with a patient complaining about hair loss. In the case of male pattern hair loss (MPHL), the next steps will aim to provide the best possible information about MPHL as a chronic progressive regression phenomenon. This includes fluctuations of its natural course, the methods for its measurement and finally the treatment options. During the lecture, we will present recent information on the practical use of non-invasive methods including global and analytical imaging methods.

Measurement of the continuum of hair restoration is still in its infancy. Who thinks about scalp hair follicles in terms of pharmacodynamic responses? Which functional characteristics in a particular target are of prognostic significance or indicators of potential for therapeutic response? What are the minimal changes of a given parameter that are clinically significant in a before – after evaluation e.g. a drug or a surgical procedure? Are the pharmacodynamic responses correlated with clinically relevant endpoints like: do the patients perceive decreased hair loss? are they satisfied in case of measurable regrowth? is a single variable or a cluster of parameters a valid surrogate for patient global satisfaction?

WS-1-D
Pathology of Pattern Hair Loss
Speaker: Wilma Bergfeld
The Cleveland Clinic Foundation, Cleveland, OH, USA

The histopathology findings correlate to the degree of patterned hair loss variation. The most characteristic finding is a progressive miniaturization of the terminal hair follicles with reduction in follicular size and normal density. Later there is loss of follicular density. There may be histological evidence of a telogen effluvium. With progression, there are increase in vellus hair follicles which results in a terminal: vellus ratio of 2:1 in contrast to normal ratio of 7:1. With miniaturization, there is a diminished size of the dermal papillae, a 30-50% reduction from normal. With progression, there is a decrease in anagen follicles and increase in telogen follicles, a reversal of the anagen telogen ratio. Other features include emptied widened follicular fibrosis tracts Elastic tissue highlights the Araro-Perkins body, a clusters of elastin, at the site of previous dermal papilla within the fibrous tract which identifies previous follicular cycles.

A shorten anagen growth cycle increases the miniaturized telogen follicles. In patterned alopecia, 83% anagen and 17% with telogen follicles as compared to normal scalp 93.5% anagen and 6.5% telogen.

In oily scalps, there is an increase the size of sebaceous glands and frequently secondary seborrheic dermatitis. Chronic lymphocytic folliculitis and or emptied inflamed fibrosis tracts, are observed in 75% with a 5% diffuse follicular and interfollicular fibrosis.
Patients with scalp hair loss seek medical attention for a many different reasons. Some patients are upset psychologically and want treatment. Others worry that their hair loss is a sign of an internal problem and simply want reassurance. As dermatologists, specialists in hair diseases, it behooves us to educate patients on the nature, course and available treatment options for a specific hair loss condition. The most common problems encountered are pattern hair loss, telogen effluvium, alopecia areata and cicatricial alopecias. Practical algorithmic approaches for management will be presented.

**Male pattern hair loss (MPHL):** Topical minoxidil solution and oral finasteride are the only treatments for MPHL that have been approved by FDA. Use is indicated in males over age 18 with mild to moderate MPHL. Well-controlled studies have proven efficacy. Stabilization, or increased scalp coverage is seen with either agent by 3-6 months of treatment and is evident by 1 year. Early intervention enhances outcome. In responders, treatment must be continued indefinitely to maintain benefit. Stopping treatment results in a return to pre-treatment status by 6 months with minoxidil and by 12 months with finasteride.

**Female pattern hair loss (FPHL):** Two percent topical minoxidil is the only FDA-approved medication for treatment of FPHL. Use is indicated in women over age 18 with mild to moderate hair loss (Ludwig stage I or II). Results may be less impressive in those with underlying hyperandrogenism.

**WS-1-F**  
**Surgical Management of Pattern Hair Loss**  
**Speaker:** Ken Washenik, MD, PhD  
**Bosley Medical Group, Beverly Hills, CA, USA**

[Abstract/summary not available at the time of printing]

**WS-1-G**  
**In Search Of Therapies For Pattern Hair Loss:**  
**Defining The Challenges And Searching For Solutions**  
**Speaker:** Satoshi Itami  
**Osaka University, Osaka, Japan**

[Abstract/summary not available at the time of printing]
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WS-2-B
Histopathology of Cicatricial Alopecia
Speaker: David A. Whiting, MD
Baylor Hair Research and Treatment Center, Dallas, TX, USA

The most common primary cicatrical alopecias are lichen planopilaris, central centrifugal cicatricial alopecia, folliculitis decalvans, discoid lupus erythematosus and nonspecific cicatrical alopecia (including pseudopelade).

The end-stage scarring from all these conditions results from the destruction and fibrosis of hair follicles, perhaps leading to polytrichia, and is frequently nonspecific on histopathologic examination. Microscopic examination of 4mm punch biopsies from early stage disease in active spreading areas of hair loss may show diagnostic changes which support a definitive diagnosis:

Lichen Planopilaris: A lichenoid interface dermatitis is common at the dermoepidermal junction of follicular infundibulum and isthmus. Peri-infundibular hypergranulosis may be seen. Sebaceous epithelium is lost in early lesions. A perifollicular lymphohistiocytic infiltrate with cytoid bodies may be present, but perieccrine infiltrates and epidermal and dermal mucin are absent. Concentric, lamellar fibrosis ensues, with pigment incontinence.

Central centrifugal cicatrical alopecia: Lymphocytic infiltrates occur around blood vessels, lower infundibulum and upper isthmus. Sebaceous epithelium is lost early on. Premature desquamation of the inner root sheath may occur. Progressive thinning of the external root sheath may cause follicular distention, rupture, hair granulomas and dense fibrosis.

Folliculitis decalvans: Acneiform dilatation may result in follicular rupture and abscess formation. The inflammation is initially neutrophilic, but is later lymphohistiocytic with plasma cells and foreign body giant cells. The process is primarily peri-infundibular, but may extend to involve the entire follicle, resulting in perifollicular fibrosis.

Discoid lupus erythematosus: A vacuolar interface dermatitis may involve surface and infundibular epidermis. Lymphocytic inflammation is prominent around hair follicles, eccrine glands between follicles and superficial and deep blood vessels. Moderate dermal mucin is present. Concentric lamellar fibrosis and pigment incontinence occur. A positive immunofluorescent LE band is present in 70% of cases.

Nonspecific cicatrical alopecia: Late stage changes from many causes include a flattened epidermis, scattered, sparse, perivascular lymphocytic infiltrates and dense fibrosis, often involving whole follicular units. A specific diagnosis is often not possible, let alone treatment.

Conclusion: Scalp biopsies are mandatory when the slightest sign of follicular destruction is found in the patient.

WS-2-C
Etiology of Cicatricial Alopecia: Background and Hypotheses to Test
Speaker: Kurt Stenn, MD
Aderans Research Institute, Philadelphia, PA, USA

The commonly recognized features of the primary cicatrical alopecias is clinically, loss of hair shafts and follicular markings and histologically, paucity to absence of sebaceous glands, presence of extrafollicular hair shafts, foreign body inflammatory reactions and follicular track fibrosis. Whatever pathogenesis one might consider must account for these widely recognized cardinal changes. Clearly, to have a hair shaft outside of the outer root sheath in the surrounding perifollicular stroma implies that a follicular implosion or explosion has occurred. In either case, a breach resulted in the outer root sheath, locally or focally, releasing the hair shaft into the dermis and inducing a foreign body reaction; the latter repairing with a focal scar. The result of this process is ablation of the whole hair follicle.

While the focus of our study must be on the elements causing follicular implosion or explosion, the lethal injury is suffered here by the mesenchyme. It is axiomatic in reparative systems that epithelium repairs and regenerates itself readily (as long as its framework is retained) while mesenchymal tissues repair with scar – a tissue with a very different function from the native supportive stroma. This principle would appear to be applicable to the hair follicle as well considering the powerful role the mesenchyme plays in inducing and supporting hair growth from various epithelial platforms. By this argument the vulnerable target in the pathway to a given cicatricial alopecia would be the mesenchyme.
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Two pathogenetic hypotheses have been put forth to explain the earliest injury in these disorders. The first proposes malfunction of the hair follicle stem cell compartment and the second proposes malfunction of the sebaceous gland and egress of the shaft. In the discussion these two hypotheses will be placed in perspective.

WS-2-D
CD200 Attenuates Hair Follicle-specific Inflammation in Mice
Speaker: Michael Rosenblum
Medical College of Wisconsin, Milwaukee, WI, USA
Authors: Michael D. Rosenblum1-2, Robert L. Truitt1, Jeffrey E. Woodliff1, Edit B. Olasz2, and Kim B. Yancey1.
1 Department of Pediatrics, 2 Department of Dermatology, Medical College of Wisconsin, Milwaukee, WI

Immunosuppressive molecules expressed on tissue-resident cells have the potential to regulate tissue-specific inflammation and autoimmunity. CD200 is a cell surface glycoprotein that transmits an immunosuppressive signal by ligating its receptor, CD200R. We have elucidated the expression of CD200 and CD200R in murine skin and examined the role of CD200-CD200R signaling in maintaining cutaneous immune homeostasis. CD200 was expressed on Langerhans cells (LCs) and on a subset of keratinocytes (KCs). CD200 expressing KCs preferentially localized to the outer root sheath of hair follicles (HF). CD200R was expressed on approximately one-third of freshly isolated LCs. LCs from CD200-/- mice showed a heightened state of activation. CD200 expression had a dramatic effect on protecting HF from inflammation and autoimmune attack. Grafts of syngeneic gender-matched skin from CD200-/- donors showed persistent perifollicular inflammation with heightened T cell recruitment and, ultimately, complete destruction of HFs, a phenotype resembling human cicatricial alopecia. Hair follicle destruction could be induced in a CD200-/- host by adoptive transfer of T cells from a mouse previously grafted with CD200+ skin. Our results suggest that the CD200-CD200R signaling pathway plays a role in establishing and maintaining immune homeostasis in the skin. This pathway may be especially important in attenuating HF-associated inflammation and/or autoimmunity.

WS-2-E
PPAR Gamma Deletion in Stem Cells of the Hair Follicle Causes Scarring Alopecia
Speaker: Pratima Karnik
University Hospital Case Medical Center and Case Western Reserve, Cleveland, OH, USA
Dermatology, University Hospital Case Medical Center and Case Western Reserve University, Cleveland, OH

Primary cicatricial or scarring alopecias (CA) are a group of inflammatory disorders of unknown pathogenesis characterized by permanent destruction of the hair follicle. Current treatment options are ineffective because the molecular basis for CA is not understood. Here we report that the lymphocytic CA, Lichen planopilaris is characterized by progressive loss of peroxisomes, abnormal lipid accumulation and infiltration of inflammatory cells and destruction of the pilosebaceous unit. Microarray analysis of LPP and normal biopsies identified decreased expression of genes required for lipid metabolism and peroxisome biogenesis. The expression of PPARγ, a transcription factor that regulates these processes, was significantly decreased in LPP. Specific agonists of PPARγ but not other PPAR subtypes were effective in inducing peroxisomal and lipid-metabolic gene expression in human keratinocytes. Finally, targeted deletion of PPARγ in the follicular stem cells in mice caused a skin and hair phenotype closely resembling scarring alopecia. These studies suggest that PPARγ is essential for healthy pilosebaceous units and loss of this function underlies the pathogenesis of LPP. We propose that PPARγ targeted therapy represents a new strategy in the treatment of these disorders.

*Note: this presentation is also shown as a Poster Presentation as P-265

WS-2-F
CCCA Update
Speaker: Elise Olsen, MD
Duke University, Durham, NC, USA
[To view this abstract, go to P-133]

WS-2-G
Secrets of Cicatricial Alopecias Revealed by Mouse Models
Speaker: John P. Sundberg, DVM, PhD
The Jackson Laboratory, Bar Harbor, ME, USA

Cicatricial (scarring) alopecias represent a diverse group of diseases in humans in which the end result is a follicular scar with permanent hairloss. Since human clinical cases, when
first seen by a physician, are in active or end stages of the
disease, it is impossible to investigate the fundamental
pathophysiology leading to these disfiguring entities.
Not surprisingly, inbred mice with single or multiple genetic
mutations develop similar, if not identical lesions. For those
single gene mutation models with multiple allelic variants
on different inbred backgrounds it is now possible to define
variations in disease due to background modifier genes as
well as different mutations within the gene itself. In addition
to being able to define the specific genes responsible for
cicatrical alopecias, which has already been done in many
cases with mice, it is possible to study mice from normality
to end stage disease to both document progression and
define the molecular networks underlying the disease
mechanisms involved. Studies with the asebia mutant
mice pioneered the field and led to the hypothesis that
sebaceous glands are critical in some but not all forms of
cicatrical alopecia. We have a group of mutant mice with
various defects of sebaceous glands and pilosebaceous
units which provide new tools to dissect which anatomical
defects are actually critical in the pathogenesis of cicatrical
alopecias. An overview of many of these models will be
presented. Most of these mouse models are readily available
from one or more repositories for investigation and as
preclinical models for screening new therapeutic approaches
to cicatrical alopecias and defining the pathogenesis of
these diseases.

WS-2-H
Cicatricial Alopecia: A Practical Diagnosis
and Therapeutic Approach
Speaker: Pascal Reygagne
Centre Sabouraud, Hôpital Saint Louis, PARIS

Scarring alopecia is an irreversible process with a permanent
destruction of the follicle. Scarring alopecia may be primary
or secondary, but our topic will be only cicatricial
alopecia, specially lymphocytic. The diagnosis is clinical and
histological and we present an overview of the clinical and
the histological assessment of cicatrical alopecias.

Clinically, cicatrical alopecia can be pustular or non
pustular, and nowadays the classification is based on the
predominant cellular infiltrate, but there is a correlation
between the clinical and histological aspect.

NON PUSTULAR (lymphocytic)
lichen planopilaris
discoid lupus erythematosus
pseudopelade
follicular degeneration syndrome
AGA with scarring evolution
alopecia mucinosa

PUSTULAR (neutrophilic)
folliculitis decalvans
tufted folliculitis
dissecting folliculitis
acne necrotica
keloid folliculitis
tinea capitis

Treatments are difficult, and most of them are not approved
or unauthorised. Non-pustular cicatrical alopecias are
treated with corticotherapy, hydroxychloroquine, and
immunomodulating agents. Pustular alopecias are treated
with antibiotics, and isotretinoïne for dissecting folliculitis.

Lichen planopilaris (LPP) is, in our experience, the
most frequent etiology of scarring lymphocytic alopecia.
Treatment is difficult and disappointing. Topical ultra
potent corticotherapy is our first line therapy. In the last
years we proved that antimalarial drugs, thalidomide and
acitretine a’nt effective to arrest progression of lichen
planopilaris: we treated without success 12 patients with
hydroxychloroquine. After this first open trial we tried
thalidomide on 4 other patients without any more success
but with side-effect. And it was the same no result with
acitretine: 8 cases without any success. When the disease
is very active the best treatment is oral corticotherapy, but
relapse is frequent.

A short course of oral cyclosporine has been reported
successful in treating 3 patients. We present 13 patients
with refractory LPP treated for 3 to 7 months with
cyclosporine and followed at least 6 months after stopping
the treatment. They were aged from 31 to 59 years
with a diagnosis of LPP from 1 to 19 years. Previous
treatment included topical and intralesional corticosteroids,
hydroxychloroquine (n=6), chloroquine (n=1), topical
tacrolimus (n=2) and oral corticotherapy (n=6). At the
beginning of the study, the dosage of cyclosporine was
3mg/kg/Day, increased monthly in case of non-response.
Treatment was stopped after 2 months without symptoms.
In a few patients we measured hair density on a selected
target and performed global standardized photography. We
obtained complete clinical resolution of the disease activity
at doses ranging from 250mg to 400mg, and treatment
duration of 4 to 8 months. A complete clinical response was
achieved in 8 patients (8/13=53%) and a partial response
in 2 (2/13=23%). Three patients failed to respond. Six
months after stopping cyclosporine 4 patients remained
symptom free and 6 had relapsed at 3, 3, 4, 4, 5, 6 months.
Hair count was available before and after treatment for
10 patients with stabilisation (n=5), increase (n=3) or
decrease (n=2). The two patients with a decrease were
clinically rated failure or partial response. Side effects were
minor: these included transient elevations of serum creatinine (n=4), distal paresthesia (4), and hypertrichosis (2).

On the basis of this experience, cyclosporine can be an option to treat refractory LPP, optimal efficient dosage is between 4 and 5 mg/kg/day, optimal course is 4 to 6 months, and success rate is 77% (10/13); relapse rate 6 months after stopping the course is 60%. A study versus systemic oral corticotherapy is necessary.

Chronic cutaneous lupus erythematosus (CCLE) is less frequent than LPP. Cutaneous lesions are frequent, especially on the face. Our first line therapy is hydroxychloroquine and topical corticotherapy. Second line therapy is oral corticotherapy or intralesional injections of triamcinolone acetonide. This treatment is injected every 3 or 4 weeks for 3 months and then every 6 to 8 weeks. Third line therapy is thalidomide or dapson or mycophenolate mofetil.

Folliculitis decalvans is, in our experience, the most frequent aetiology of scarring neutrophilic alopecia. Our first line therapy is doxycycline alone, 100mg twice a day for one months, then 100mg for 6 months more. Minocycline can be more effective but serious side effects are more frequent especially in black patients. Our second line therapy is the association of rifampicine 300mg twice a day with clindamycine or fusidic acid or ciprofloxacine for 10 weeks. Zinc gluconate with fusidic acid can be an option.

Dissecting folliculitis is less frequent. Isotretinoin is the best treatment. We use 0.5mg/kg to 0.75mg daily for 6 to 9 months. An antistaphylococcic antibiotherapy may avoid a flare-up during the first month of isotretinoin. In case of relapse a second cure is possible and maintenance therapy with a low dosage for many months may be necessary.

In conclusion, treatment of cicatricial alopecia is difficult. A clinical and photographic assessment is important, but the progression of the disease is slow and insidiously and an hair count assessment on a selected area is the better criterion to be sure of the stabilisation and the remission of the disease.

WS-2-I
Cicatricial Alopecia: The Future
Speaker: Rodney D. Sinclair, MBBS, MD, FACD
St. Vincent’s Hospital Melbourne, University of Melbourne, SA, Australia

Currently, the cicatricial alopecias include a poorly understood group of hair disorders linked by the common final pathway of permanent hair follicle loss and replacement by fibrous tissue in involved areas. There are no pathognomonic diagnostic tests for the primary cicatricial alopecias. There is no clear understanding of the natural history and in particular whether the primary cicatricial alopecias ever ‘burn out’. There is no tool useful for monitoring therapeutic response in multifocal disease on the scalp. There is no treatment known to arrest progression, and severely affected patients lack sufficient donor hair population for transplantation.

In the absence of published randomized controlled clinical trials to guide therapy and no clear investigational protocol for the conduct of such trials, and no clear end-points as when to ultimately cease therapy, I perceive no clear role for medical therapy.

As hair follicle neogenesis is likely to result in new hairs subject to the same fate as the one’s they replace, the best possible future outcome that I can envisage is hair cloning with implantation on demand of immunologically distinct hairs (or hair follicle stem cells) that are spared from the inflammatory attack.

Failing that, coloured hair sprays for limited disease and wigs for extensive disease are likely to dominate the foreseeable future. Hair transplantation for secondary cicatricial alopecias and non-progressive primary disease will continue to have a role.

Workshop 3
Alopecia Areata Pre Conference

Supported by an unrestricted educational grant from the National Alopecia Areata Foundation

WS-3-A
Genetics
Speaker: Angela Christiano, PhD
Columbia University, New York, NY, USA

Alopecia areata (AA) is a genetically determined, immune-mediated disorder of the hair follicle with a lifetime risk of approximately 2%, making it one of the most common autoimmune diseases. It is defined by a spectrum of severity that ranges from patchy localized hair loss on the scalp to the complete absence of hair everywhere on the body. In an effort to define the genetic basis of AA, we performed a genomewide search for linkage in 20 families with AA. Our analysis revealed evidence of at least four susceptibility loci on chromosomes 6, 10, 16 and 18, by use of several different statistical approaches. Fine-mapping analysis with additional families yielded a maximum multipoint LOD score of 3.93 on chromosome 18, a two-point affected sib pair (ASP) LOD score of 3.11 on chromosome 16, several ASP LOD scores >2.00 on chromosome 6q, and an HRR LOD of 2.00 on chromosome 6p in the region of the MHC.
WS-3-B
Mechanisms of Alopecia Areata
Speaker: Kevin J. McElwee, MD
Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada

The last ten years have seen a concerted effort to investigate the nature of alopecia areata (AA). Significant inroads have been made, but our understanding of AA remains limited. Thus far, most evidence is consistent with an autoimmune mechanism of disease development. The transfer of immune cells in disease models has demonstrated that hair loss is primarily mediated by leukocytes. However, without knowledge of the primary target antigen epitopes involved in disease initiation the putative autoimmune nature of AA remains unproven. It is generally accepted that AA probably involves a genetic susceptibility to autoimmunity and AA, but onset of actual hair loss is most likely precipitated by the interaction of genes with the environment. For some, genetics may be the greater influence on AA development while for others the environment may provide a stronger input. Several environmental factors have been suggested to influence the course of AA (such as stress, hormonal fluctuations, and infectious agents), but evidence of their true significance in disease initiation is lacking. The nature of the actual disease promoting mechanism(s) for AA onset and leukocyte targeting of hair follicles remains unknown, but several hypotheses have been suggested. Possible mechanisms involve a failure of the hair follicle's reputed immune privilege, inappropriate presentation of hair follicle antigens to the immune system during catagen regression, failure of central or peripheral immune regulation, non-specific activation of autoreactive lymphocyte clones, or antigen epitope mimicry by pathogens. Overall, fundamental questions concerning the mechanisms of AA development remain to be answered.

WS-3-C
Histopathology of Alopecia Areata
Speaker: David A. Whiting, MD
Baylor Hair Research and Treatment Center, Dallas, TX, USA

The microscopic findings in alopecia areata reflect the duration of the current episode. The characteristic, peribulbar, lymphocytic infiltrate is seen in the acute phase of a developing patch. Initially it surrounds terminal anagen hair bulbs in the lower dermis and causes anagen arrest; in recurrent attacks when most hairs are miniaturized, it may only be found around vellus-like bulbs in the upper dermis. After 3 or 4 weeks the subacute phase supervenes and is signified by the increased numbers of catagen and telogen hairs resulting from the anagen arrest, with decreasing lymphocytes. After 1-2 months the chronic phase ensues, characterized by a marked decrease in terminal hairs and a reciprocal increase in vellus hairs. Recovery is indicated by increasing numbers of terminal anagen hairs, decreasing vellus hairs and the disappearance of inflammation.

Always try and take a biopsy from the spreading edge of the most recent area of hair loss. Diagnostic difficulties arise when biopsies are taken in later stages of an attack, or in the nondescript diffuse form of the disease labeled alopecia areata incognita. High percentages of telogen and/or miniaturized hairs should arouse suspicions, and vellus hair bulbs with surrounding lymphocytes should be sought.

WS-3-D
Clinical Features
Speaker: Maria Hordinsky, MD
University of Minnesota, Minneapolis, MN, USA

CLINICAL FEATURES

Patterns
Alopecia areata may present in one of several distinct patterns:
1. Round or oval patches of hair loss
2. Loss of all terminal scalp hair (alopecia totalis)
3. Loss of all scalp and body hair (alopecia universalis)
4. Ophiasis (bandlike) pattern of hair loss
5. Reticular variant of patchy alopecia areata
6. Diffuse scalp alopecia areata
7. Perinevoid alopecia areata (rare)

Hair Fibers
1. Explanation mark hairs
2. Fibers in anagen arrest
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Pigmentation
1. Hair pigmentation is frequently affected
2. Disease prefers attacking pigmented hairs, sparing unpigmented or white fibers

Differential Diagnosis
1. Tinea capitis
2. Traction alopecia
3. Loose anagen syndrome
4. Aplasia cutis
5. Pseudopelade
6. Other

Nails
1. Frequency of nail abnormalities ranges from 10-66% 
2. May precede, follow or occur concurrently with hair loss activity 
3. Nail pitting is most commonly seen 
4. Other findings include: 
   a. Longitudinal ridging 
   b. Koilonychia 
   c. Brittle nails 
   d. Onycholysis 
   e. Onychomadesis 
   f. Periungual erythema 
   g. Other

Sweat Glands
Number and function have been reported to be normal or decreased.

Disease Associations
1. Atopy (allergic rhinitis, asthma, and atopic dermatitis) – up to 40% in some studies while the prevalence of atopic diseases in the population is estimated to be 20%. 
2. Thyroid disease 
3. Autoimmune diseases 
4. Patients seropositive for the human immunodeficiency virus. 
5. Type 1 diabetes mellitus 
   Interestingly, there is reportedly more diabetes present in the relatives of patients with alopecia areata but not in patients themselves, suggesting that the predisposition of alopecia areata may be protective against the development of diabetes. 
7. Autoimmune Polyglandular Syndrome (APS-1), chronic hypoparathyroidism-mucocutaneous candidiasis-autoimmune adrenal insufficiency) – up to 30 percent of affected patients may express alopecia areata.

8. Unusual associations: 
   • Testicular atrophy or dysfunction 
   • Ophthalmologic changes such as iris color change, pigment hyperplasia of the choroid and retinal epithelium

WS-3-E
Alopecia Areata – Topical Immunotherapy 
Speaker: Jerry Shapiro, MD
University of British Columbia, Vancouver, BC, Canada

There are no FDA-approved treatments for alopecia areata (AA). Corticosteroids, contact immunotherapy, topical minoxidil, and psoralen combined with UVA irradiation (PUVA) comprise the more commonly used modalities. None are curative nor reliably effective. Recommendation for use of any one therapeutic agent is hampered by the lack of long-term, randomized, double-blind, placebo-controlled studies of sufficient power and duration to determine efficacy unequivocally. This is further compounded by the failure of many studies to control for rates of spontaneous remission or to stratify patients according to type of disease, among other prognostic factors. Because most patients resolve within 1 year without any intervention, half-head/half-lesion studies are essential to establishing treatment effect. Precise definition of “response”, often omitted, aids in interpretation of results. Despite this complexity, there are a number of treatments that do appear to work in some patients with AA. Topical immunotherapy has been shown to be effective in almost 70% of non-totalis patients. Seventeen percent of totalis patients respond to therapy. Half however relapse overtime and no longer respond to therapy. Side effects include dyspigmentation, eczematous dermatitis and lymphadenopathy.

WS-3-F
Biologics, Calcineurin Inhibitors and What’s Ahead in Alopecia Areata 
Speaker: Vera Price, MD
University of California San Francisco, San Francisco, CA, USA

Alopecia areata (AA) is a T cell-mediated autoimmune disease in which anagen hair bulbs are targeted by CD4+ and CD8+ lymphocytes. The initiation phase of AA is mediated by type 1 cytokines including interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, all of which are expressed in lesional AA skin. The immunologic basis of alopecia areata is similar, though not identical, to that of psoriasis. Knowledge of the immunologic basis of psoriasis has resulted in creation of genetically engineered biologic agents that target the key pathogenic steps, primarily those mediated by T cells and the inflammatory cascade. These
agents include those that decrease the number of activated T-cells (alefacept), those that block T-cell activation, binding, and trafficking to the dermis and epidermis (efalizumab), and those that specifically bind tumor necrosis factor-alpha (etanercept and infliximab). Biologic therapies target specific cell surface receptors, and their potential advantage is that their greater specificity provides better safety profiles. These four biologic agents have been approved by the US FDA for the treatment of psoriasis. Because previous clinical experience has suggested that treatments effective in psoriasis may also be effective in AA, biologics have been used in alopecia areata. This report includes the use of efalizumab in the first randomized, double-blind, placebo-controlled study in patients with AA. It also highlights the importance of controlled studies to assess efficacy of treatments in AA. So far, the use of biologics in alopecia areata has not shown efficacy. In fact, during the course of treatment with anti-TNF agents, patients have developed alopecia areata. The experience with biologics, calcineurin inhibitors, and future directions in alopecia areata, are the subject of this report.

References

Alefacept is a fusion protein that blocks T-cell activation. Efalizumab is a humanized monoclonal antibody that binds to CD11a, a component of LFA-1 that binds to ICAM-1 on antigen presenting cells. Etanercept is a human fusion protein and infliximab is a chimeric (mouse/human) antibody and both inhibit the inflammatory cytokine TNF-alpha.

Hyperproduced interferon-gamma has been postulated as a key mediator of autoimmune disease. It is expressed in lesional AA skin, and interferon-gamma knockout mice are resistant to the development of AA.5 A small, open, pilot trial of anti-interferon-gamma antibodies in patients with active AA has shown promise as an early intervention for this disease.

Keywords: alefacept, efalizumab, etanercept, and infliximab

Workshop 4
Hirsutism Pre Conference

WS-4-A
Aetiology of Hirsutism
Speaker: Valerie A. Randall, PhD
University of Bradford, Bradford, United Kingdom

Hirsutism, or male pattern hair distribution in women, causes psychological distress. This is because the main role of the additional hair developed during, and after, puberty focuses on social and sexual communication. Pubic and axillary hair signals adulthood in both sexes, while beard, chest and other body hair identify the sexually mature male. Therefore, hirsutism may make a woman think she is turning into a man.

Most patients with hirsutism present with terminal (readily visible, pigmented) hair on the moustache and chin areas; this is often accompanied by hair on the chest, abdomen and thighs. The amount of increased hair that is unacceptable varies with cultural/ethnic background. Hirsutism may be associated with other signs related to androgens including acne or androgenetic alopecia or with acanthosis nigricans (dark patches of skin).

Androgens are the normal stimulant for adult human hair growth patterns and are strongly implicated in female hirsutism. Hirsutism can be caused by endocrinological changes including increased androgen production by the adrenals or ovaries and is often associated with Polycystic Ovarian Syndrome. Sudden, dramatic onset requires urgent endocrine investigation as it may indicate an androgen-secreting adrenal or ovarian tumour. Although raised circulating androgen levels or low sex hormone binding globulin levels are common, some hirsute women do not show obvious causes i.e. are idiopathic. This is likely to involve an increased sensitivity to normal androgens within the hair follicles themselves. Greater understanding of how androgens act within the hair follicle should lead to better treatments for hirsutism.
Invited Speaker Presentations

Wednesday, June 13, 2007

WS-4-B
Presentation and Evaluation of Hirsutism
Speaker: Ulrike Blume-Peytavi, MD
Clinical Research Center for Hair and Skin Physiology – CRC, Charité – Universitätsmedizin Berlin, Berlin, Germany

For many women excessive facial hair growth may have devastating consequences with impact on self image, loose of self confidence, loose of their femininity, hindering them in social contacts. It is a condition which can occur from puberty to adulthood, in menopause and even later. Profound exploration of the patient's history has to be performed, when there is any evidence of gynaecological problems the patient should be referred to an endocrine gynaecologist.

Androgen excess may have profound effects on the skin, soma and psyche of patients presenting clinical signs of peripheral hyperandrogenism. It is one of the most common disorders affecting 10% of adult women before the menopause. Most of these women are suffering from acne, alopecia, seborrheoa, hirsutism, menstrual disturbances, anovulation, insulin resistance and obesity. Virilisation is unusual except in patients with ovary or adrenal cancer. More than 90% of hyperandrogenism is due to polycystic ovary syndrome (PCOS) and idiopathic hirsutism. Less frequently suprarenal and ovarian malignant tumors, or drug induced hyperandrogenism can be observed. The latter is mainly due to uptake of anabolic steroids, testosterone, and contraception with long-acting progestogene, long-term corticosteroid or cyclosporine therapy.

Polycystic ovary syndrome (PCOS) is a complex phenotypic spectrum of primarily hyperandrogenic signs and symptoms. Common dermatologic manifestations of PCOS include hirsutism, acne, acanthosis nigricans, and androgenic alopecia. Hirsute women often have increased activity of 5α-reductase, the enzyme that converts the androgen testosterone to its active metabolite, in hair follicles. Likewise, androgens affect the formation of acne by increasing sebum production from sebaceous glands in the skin.

Androgen metabolism occurs within the pilosebaceous unit, as recently demonstrated by the presence of local differences in the amounts of aromatase, 5α-reductase as well as the androgen receptor (AR). These steroid metabolising enzymes convert weak to more potent androgens, underscoring that the skin is an endocrine target tissue for androgen hormone action, similar to ovaries, testes, and adrenal glands.

Today, peripheral signs of hyperandrogenemia demand an extensive work up of the underlying cause and often present a challenge to the dermatologists in achieving successful management.

WS-4-C
Hirsutism in Children
Speaker: Danielle Marcoux, MD
CHU Sainte-Justine, University of Montreal, Quebec, Canada.

In prepubertal children, hirsutism will manifest with terminal hair over androgen-dependant areas, usually pubis, axilla, labia majoris or base of the penis. When more severe, and in older children, terminal hair will be present over other androgen – dependant areas, such as the upper lip, chin, jaws, neck, internal thighs, buttocks etc.

Idiopathic hirsutism is the most frequent cause, and is usually familial and ethnic. In prepubertal children, hirsutism with precocious puberty is the most frequent pathological cause and may manifest with isolated adrenarche and pubarche with pubic and axillary hair, body odor and acne. Polycystic ovary syndrome and its variant, hyperthecosis, with or without insulin resistance, may first manifest itself in childhood with hirsutism as an early manifestation. As in adults, serious tumoral or non-tumoral virilizing disorders are extremely rare, account for less than 1% of the etiologies, and may present with cliteromegaly or penile enlargement as well as hirsutism and acne.

Important and sensitive signs of precocious puberty are acceleration of the growth curve pattern with tall stature, advanced bone age and signs of hyperandrogenism.

WS-4-D
Treatment of the Hirsute Patient
Speaker: Wilma Bergfeld
The Cleveland Clinic Foundation, Cleveland, OH, USA

Hirsutism can be seen in females and males. In females it represents unwanted terminal hair in a male distribution while in males it manifests as excessive male body and facial hair. Hirsutism in both sexes has a genetic basis and is related to 1) excess in circulating androgens (organ hormonal abnormalities or over production) or 2) target organ excess (peripheral androgen metabolism) of androgens with in the hair follicle and sebaceous gland. There are multiple potential defects of androgen production, transport, metabolism, and clearance. The majority of patients have a genetic basis for their hirsutism unless a tumor, endocrine cancer or androgenic drugs are identified as the inducer. The first step in treatment is to evaluate the etiology of the hirsutism. Once the androgen defect is identified then a targeted therapy can be employed.
Targeted therapies include hormonal suppressive therapies, androgen agonists, androgen receptor antagonists, 5-alpha reductase inhibitors, insulin sensitizing agents, weight reduction, and surgery. Topical therapies include enzyme inhibitors of hair growth, physical and chemical removal of hair. These therapies will be discussed. Commonly combined and prolonged therapies are required.

**WS-4-E**  
**Lasers and the Future**  
**Speaker:** R. Rox Anderson, MD  
*Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, USA*

“Permanent hair reduction” by laser or intense pulsed light treatments is safe and very effective for hirsute women with dark coarse hair, with some important exceptions. Significant problems include lack of response of white hair, lack of knowledge about combination with medical treatment and infrequently, paradoxical apparently permanent hair stimulation. Stimulation tends to occur in women of Mediterranean and near eastern descent, with a phenotype of fine dark facial hair and an ill-defined frontal hairline. Safe treatment of dark skin with longer wavelength, longer pulse duration devices, with skin cooling and appropriate fluence will be discussed. Repeated photothermal damage of hair follicles at relatively low laser fluence induces a catagen-like state that can be used for hair growth control. In the US, FDA recently cleared a laser hair removal device for home use. Photodynamic therapy with topical aminolevulinic acid (ALA-PDT) and high fluence red light has been shown to inactivate anagen hair follicles and sebaceous glands, but has not been developed as a treatment for hirsutism. ALA-PDT may be superior to laser treatment by targeting both acne and hair, regardless of hair color. Advanced laser microscopy of skin, e.g. by optical coherence tomography, can clearly image whole hair follicles in vivo. Potentially, imaging will allow better assessment of response to experimental or routine therapy, and might also be combined with a laser microbeam scanner to provide “robotic” treatment of hirsutism.

**Thursday, June 14, 2007**

**S-1-A**  
**Cancer-Initiating Cells: From Leukemia to Solid Tumors**  
**Speaker:** John E. Dick, PhD  
*University Health Network and University of Toronto, Toronto, ON, Canada*

Two fundamental problems in cancer research are identification of the normal cell within which cancer initiates and identification of the cell type capable of sustaining the growth of the neoplastic clone. There is overwhelming evidence that virtually all cancers are clonal and represent the progeny of a single cell. What is less clear for most cancers is which cells within the tumor clone possess tumor initiating or “cancer stem cell” (CSC) properties and are capable of maintaining tumor growth. In the last decade there has been progress to identify the CSC of some hematologic and solid cancers. If CSC lie at the heart of cancer, then the biological properties of CSC provide a foundation for the development of more effective therapies. Studies of human acute myeloid leukemia are the most advanced and this session will review the current state of knowledge of leukemic stem cells. A major finding was that LSCs are not functionally homogeneous but, like the normal hematopoietic stem cell (HSC) compartment, comprised of distinct hierarchically arranged LSC classes. In addition, studies will be described on the development of robust experimental models whereby normal human stem and/or progenitor cells can be transformed into full-blown leukemic cells. This approach provides a significant step forward to understand the mechanisms involved in human leukemogenesis and the rules for converting normal hematopoietic cells into leukemic cells. Finally, recent work on the identification of the CSC in colon cancer will be discussed.

**S-1-B**  
**Hair Follicle Stem Cells – Epithelial**  
**Speaker:** George Cotsarelis, MD  
*University of Pennsylvania, Philadelphia, PA, USA*

Over 15 years ago, we proposed that quiescent keratinocytes in the hair follicle bulge were epithelial stem cells important for hair follicle cycling, epidermal renewal, wound healing and carcinogenesis. Since that time, we identified cytokeratin 15 (K15) expression as a marker for these cells and developed several transgenic mouse models using the K15 promoter to further study the bulge cells. Using K15-EGFP mice, we isolated bulge cells and demonstrated that they possessed an epithelial stem cell phenotype of quiescence, high proliferative
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potential and multipotency. We also characterized the cells at the molecular level using microarrays and identified approximately 150 differentially expressed genes in these cells. Through genetic lineage analysis using an inducible K15-CrePR;R26R bigenic mouse, we showed that bulge cells generate all of the epithelial lineages within the lower anagen hair follicle. However, ablation of bulge cells using K15-thymidine kinase mice resulted in permanent hair loss but survival of the epidermis. Over a prolonged period, bulge cells did not contribute to epidermal homeostasis, but in response to wounding bulge cell progeny rapidly moved into the wound area to assist in reepithelialization. Bulge derived cells did not persist in the epidermis indicating that epidermal stem cells and hair follicle stem cells are distinct populations each with self renewing capabilities.

Session 2
Mesenchymal Stem Cells

S-2-A
Skin-derived Precursors (SKPs) and Induction of Hair Follicle Morphogenesis
Speaker: Freda Miller
Hospital For Sick Children, University of Toronto, Toronto, ON, Canada

We have previously isolated and characterized a population of neural crest-related precursors from rodent and human dermis termed SKPs for skin-derived precursors. SKPs can differentiate into a number of neural crest-derived cell types including myelinating Schwann cells, bone and cartilage. This talk will focus upon our recent work asking about the endogenous role of SKPs within the dermis. We demonstrate that SKPs, when transplanted into adult skin will reconstitute the dermal components of skin, but not the epidermal. Moreover, SKPs can induce de novo morphogenesis of hair follicles, where they comprise both the dermal papilla and dermal sheath. Thus, SKPs may represent a dermal stem cell that maintains both inductive and differentiation potential throughout adult life.

S-2-B
Manipulating Gene Expression in the Dermal Papilla of the Mouse in vivo
Speaker: Bruce A. Morgan
CBRC, Charleston, MA, USA
Authors: David Ensell-Seijffers and B.A. Morgan

The remarkable progress in hair biology in recent years has been fueled in part by the ability to manipulate gene expression in the stem cells and other keratinocytes of the follicle in vivo and to exploit this ability to purify these populations for in vitro analysis. However, the behavior of these keratinocytes during follicle morphogenesis and cycling is dependent on interactions with the dermal papilla, a mesenchymal component of the follicle. The abilities of dermal papilla to drive de novo follicle formation and to direct morphogenesis of the cycling follicle make these cells an attractive target for either cell based or pharmacologic approaches to augmenting or restoring hair growth. Despite this fact, DP cells remain poorly understood when compared to the keratinocyte constituents of the follicle. To facilitate research on this population, we have developed mouse strains that express cre recombinase specifically in the dermal papilla of the hair follicle. These allow the manipulation of gene expression in the DP of existing hair follicles. The use of these mice to study gene activity and other aspects of DP cell behavior will be discussed.

S-2-C
Mesenchymal-Epithelial Interactions Needed for Tissue Engineering of Hair Follicles
Speaker: Colin Jahoda
University of Durham, Durham, United Kingdom

[Abstract/summary not available at time of printing]

Session 3
Tissue Engineering

S-3
Tissue Engineering of Hair Follicles
Speaker: Kurt Stenn, MD
Aderans Research Institute, Philadelphia, PA, USA

Session 4A
Hair Surgery

S-4A-a
Update on Concepts and Techniques in Hair Transplantation 2007
Speaker: Walter Unger
University of Toronto, John Hopkins Medical School, Toronto, ON, Canada

Objectives: The objective of the presentation will be to not only present current concepts and techniques in hair restoration surgery for men and women, but also to briefly describe the pros and cons of some of the more controversial and sometimes vigorously promoted aspects of the procedure. The latter includes follicular unit extraction (FUE), “megasessions” of more than 2500 FU/session and “dense packing” of FU (over 35 FU/cm²). The appropriate
stage/age at which one can reasonably undergo hair transplanting will also be discussed.

**Approach:** Techniques and results will be clarified through the use of a large number of photographs. Controversies will be dealt with on the basis of tabulated results of studies that have been done to date.

**Results:** Current techniques in hair restoration surgery are varied, but each can result in excellent cosmetic results. Long-term consequences of megasessions and dense packing make their use less desirable in younger individuals in whom the long-term prognosis is less certain. FUE rather than conventional harvesting of graft material is appropriate only in a relatively small subset of individuals.

**Conclusion:** Hair restoration surgery continues to evolve with hair survival and results improving almost yearly. Long-term consequences of the results of recent innovations have yet to be determined and therefore their cautious utilization in properly selected individuals is advocated.

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**S-4A-b**

**Evidence Based Hair Restoration**

**Speaker:** Andreas M. Finner, MD  
Otto-von-Guericke-University Magdeburg, Hair Clinic, Magdeburg, Germany

In spite of recent advancements in technique and aesthetic results, the evidence based on randomized controlled trials (RCT) is limited in hair transplantation (HT). This is partly due to general problems when assessing hair growth, but there are also unique problems when conducting trials in HT.

However, it is imperative to increase the level of evidence in HT. Potential factors that initially need to be investigated include 1) the dynamics of graft hair growth after the procedure, 2) the extent of shock loss and 3) the survival rate of grafts. Concomitantly, efficacy of various techniques and perioperative management can be investigated.

The International Society of Hair Restoration Surgery has formed a task force for evidence based medicine (EBM) to create guidelines for HT studies, increase motivation for surgeons and patients, define important research topics and promote multi-center trials. The members of this task force include both hair researchers and surgeons.

As a first step, we will initiate one large multi-center RCT to evaluate graft survival under standardized conditions. In future studies, hair thickness may become a representative parameter. There are some initial data indicating that the effect of HT does not only result from adding hairs. Pre-existing vellus and miniaturized hair may merely be partially replaced by thicker terminal hairs of higher quality.

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**S-4A-c**

**Racial Differences in Hair Transplantation**

**Speaker:** Valerie Callendar  
Howard University College of Medicine, Washington, DC, USA

Due to the changing demographics in the United States, people of color will represent almost 50% of the population by 2050. Therefore, more patients of color will be presenting to the physician seeking medical and surgical treatment for their hair loss. Hair transplant surgery is a common cosmetic procedure used to correct hair loss in men and women of all races and ethnicities. However, there are racial differences and therapeutic challenges which must be addressed when performing hair transplant surgery in patients of color and a clear understanding of these differences allows a greater success in this population of patients. Although there are no major biochemical differences among black, Caucasian, and Asian hair types, there are apparent differences in the hair morphology and hair densities among racial groups. In most cases, the hair structure of black hair is tightly coiled and the hair follicle curved, which can be challenging to the surgeon in donor harvesting and graft preparation. Furthermore, indications, contraindications, surgical instrumentation, preoperative and postoperative counseling will vary as well. In addition, there is an increase risk of hypertrophic scarring and keloid formation in patients of African descent. Finally, in patients with central centrifugal cicatricial alopecia, an inflammatory form of scarring alopecia, there are special considerations that must be addressed when performing hair transplantation. These include alterations in the hair grooming practices, aggressive medical therapy, a test session with biopsy to confirm the absence of inflammation prior to the surgical procedure and a decrease survival rate of transplanted grafts.
Invited Speaker Presentations

Thursday, June 14, 2007

S-4A-d
Hair Restoration Surgery in Unusual Cases: Cicatricial Alopecia, Congenital Alopecia and Other Alopecias
Speaker: Nilofer P. Farjo
_Farjo Medical Center, Manchester, United Kingdom_

The vast majority of hair restoration procedures are performed for androgenetic alopecia. Transplanting into areas where there is alopecia due to an inflammatory disease process is considered to be contraindicated. However, there are certain cases where the disease process has 'burnt out' when it may be appropriate to transplant after first ascertaining that the disease has not progressed for a minimum time period and biopsy is negative for inflammation. In these situations the clinical course can be relapsing and remitting so we can never be sure that the disease will not reactivate in future. The patient may, however, be grateful for a temporary cosmetic improvement. Congenital aplasias are also situations in which transplantation may be considered if donor hair is adequate.

I will present a number of cases where the decision to transplant was taken after biopsy and clinical evidence pointed to a quiescence of the disease process. Our criteria for transplantation is:

1. No clinically event disease progression for 5 years
2. Biopsy negative
3. Sufficient donor hair for adequate coverage
4. Patient understands that the disease may recur and there may be decreased hair survival
5. Evaluation of the possible development of or progression of concurrent androgenetic alopecia

In some cases where the nature of the scarring indicates the possibility of poor growth test grafting procedure was employed.

Two techniques were employed in the surgical treatment of the patients: scalp reduction and follicular unit grafting. Cases selected included diagnoses such as lichen planus, ulerythema pyogenes, triangular alopecia. Surgical results are presented with the minimum follow up period of 6 months. Some long term results are available. In selected cases surgical restoration of hair in conditions generally considered to be contraindicated can be performed.

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Session 4B
Pathology: Animal and Human Pathology

S-4B
Histopathology of Selected Mouse Models for Human Hair Diseases
Speakers: David Whiting, MD¹,
Magdalena Martinka, MD², and
John P. Sundberg, DVM, PhD³

¹Dallas Associated Dermatologists, Dallas, Texas;
²Dept. Pathology, University of British Columbia, Vancouver, BC; ³The Jackson Laboratory, Bar Harbor, Maine, U.S.A.

While there are obviously big differences in size, hair type and quantity, color, and shape of mice and their hair follicles and fibers when compared to humans, the fundamental anatomy, biology, genetics, and diseases are remarkably similar. Repositories around the world make mouse models readily available for investigators to study many diseases. While not all of the human hair diseases have a known genetic basis, many do and homologous models exist. Drs. Whiting and Martinka will present a series of interesting clinical cases from their respective practices and Dr. Sundberg will attempt to match these, where possible, with similar or identical mouse models. Glass slides and microscopes will be provided in advance as well as after the session. Discussions will focus on gross and histologic features of the hair follicle diseases with a brief summary of the genetics and underlying pathophysiology, if known.

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Session 4C – Congenital Hair Loss/Ectodermal Dysplasia

S-4C
Review of Ectodermal Dysplasias
Speaker: Angela Christiano, PhD
_Columbia University, New York, NY, USA_

[Abstract/summary not available at time of printing]
Invited Speaker Presentations

Friday, June 15, 2007

Session 5
Morphogenesis / Follicular Cycling

S-5-A
Ebling Lecturer: Wnt and Notch Signaling Pathways in Development and Cancer of the Gut
Speaker: Hans Clevers
Hubrecht Laboratory, Utrecht, The Netherlands

Mutations in the Wnt pathway components APC, beta-catenin and conductin all induce sustained complex formation of the co-activator beta-catenin with TCF transcription factors. The resulting transactivation of TCF target genes represents the primary transforming event in colorectal cancer (CRC). Yet, the consequence of the presence of mutationally activated beta-catenin/TCF in fully transformed CRC cells is unknown. We have constructed CRC cell lines carrying inducible dominant-negative TCF constructs. Inhibition of beta-catenin/TCF resulted in a rapid G1 arrest. DNA array analysis revealed the downregulation of a small set of transcripts. These genes were expressed in polyps, but also, physiologically, in the crypt progenitor compartments of the colon. Beta-catenin/TCF thus imposes a crypt progenitor phenotype on CRC cells. Moreover, inhibition of beta-catenin/TCF activity restores the differentiation program, despite the presence of multiple other mutations in CRC.

TCF target gene expression is always restricted to the crypt, but target genes can be sub-classified based on expression patterns within the crypt. We have tentatively identified at least three target genes which are expressed uniquely in the crypt stem cells.

The Wnt cascade is not the only signaling pathway controlling cell fate along the crypt-villus axis. Upon blocking the Notch pathway genetically, we observe a massive conversion of proliferative crypt cells into post-mitotic goblet cells. A similar phenotype was obtained by blocking the Notch cascade using a gamma-secretase inhibitor. The inhibitor also induced goblet cell differentiation in intestinal adenomas. Our data imply that gamma-secretase inhibitors, developed for Alzheimer disease, may be of therapeutic benefit in colorectal cancer.

S-5-B
New Insights into Telogen
Speaker: Cheng Ming Chuong, MD, PhD
University of Southern California, Los Angeles, CA, USA

In a population, hair follicles can go through regenerative cycle autonomously, simultaneously, or by coupling to generate waves, resulting in complex living hair cycle domains. Here we show each domain consists of initiation sites, propagating wave, and boundaries. Boundaries form because waves hit follicles which are refractory. Using hair plucking, we can define refractory and competent telogen follicles. Molecularly, refractoriness is characterized by expression of Bmp pathway members intra- and inter-follicularly. A BMP reporter mouse sums up oscillating BMP activities in vivo. KRT14-NOG mice shows minimal refractory telogen and simplified transverse wave dynamics. Mutated skin flap transplanted to a normal host exhibits non-autonomous interactions and partial rescue of refractory telogen. A mathematical model based on cellular automata is developed to simulate the behavior of regenerative waves. This novel systematic approach shows sequential re-entry of hair regeneration depends on the stochastic equilibrium among follicle stem cells, intra-follicle micro-environment, and inter-follicle macro-environment.

S-5-C
Wnt Signaling in the Control of Hair Follicle Development
Speaker: Sarah E. Millar, PhD
University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Wnt/β-catenin signaling is required for embryonic hair follicle induction, and K14 promoter-driven expression of stabilized β-catenin causes ectopic hair follicle formation postnatally, suggesting a key role for this pathway in determining hair follicle fate. However the consequences of forced activation of β-catenin signaling in embryonic mammalian skin are not clear. To address this question we mutated endogenous β-catenin to a dominant active form in embryonic surface ectoderm. Hair follicle placode induction was markedly accelerated in mutant embryos, but the placodes failed to invaginate. Wnt reporter gene activation was widespread in E14.5 mutant skin, unlike its normal pattern in developing placodes. Analysis at E17.5 revealed a global failure of epidermal differentiation in the mutant, indicated by lack of expression of the suprabasal and terminal differentiation markers K10, involucrin and loricrin. Instead, the surface epithelium broadly expressed the hair shaft cortex marker AE13. Consistent with global adoption of hair follicle fate, the molecular regulators of hair follicle development Bmp2, Shh and Edar were expressed broadly in mutant epithelium, and the follicular dermal condensate markers Bmp4 and alkaline phosphatase were expressed throughout the upper dermis. Hair follicle outer and inner root sheath markers were not expressed, indicating specific adoption of hair shaft cortex fate by mutant epithelial cells. These data demonstrate that Wnt/β-catenin signaling is a master regulator of cell fate.
in embryonic surface ectoderm, suppressing squamous differentiation and activating hair follicle initiation and cortex-like differentiation. In addition, both formation of a patterned array of hair follicle placodes and hair germ invagination require controlled downregulation of β-catenin signaling.

**Session 6**

**Follicular Growth Controls**

**S-6-A**

**Controlling Hair Follicle Morphogenesis Through Polyubiquitination**

**Speaker:** Anthony E. Oro, MD, PhD  
**Stanford University, Stanford, CA, USA**

**Authors:** Anthony E. Oro and Erik Huntzicker, Program in Epithelial Biology, Stanford University School of Medicine

Hair follicle development and maintenance require precise reciprocal signaling interactions between the epithelium and underlying dermis. Several key developmental signaling pathways including Wnt, Shh, BMP, and NFκB/Edar are indispensable for this process and when aberrantly activated, can lead to skin and appendage neoplasms. Recent data point to protein polyubiquitination as playing a central role in regulating the timing, duration and location of signaling. Here we focus on how polyubiquitination regulates one of the signaling pathways, Shh, and demonstrate two distinct destruction pathways are required to regulate the Gli activators and a distinct pathway to regulate Gli3 repressor. We find that at least one of the destruction complexes that regulates Gli stability is shared with both the Wnt and NFκB pathway, arguing that polyubiquitination may be a critical global mechanism for controlling the duration and intensity of the hair cycle.

**S-6-B**

**Hedgehog Functions in the Pilosebaceous Unit**

**Speaker:** Andrzej Dlugosz, MD  
**University of Michigan, Ann Arbor, MI, USA**

**Authors:** Andrzej Dlugosz, Hong Sheng, and Mark Hutchin – University of Michigan Department of Dermatology, Ann Arbor, MI, USA

The discovery of loss-of-function PTCH mutations in Neviod Basal Cell Carcinoma Syndrome provided the first evidence linking the Hedgehog (Hh) signaling pathway to basal cell carcinoma (BCC) development. PTCH, a receptor for secreted Hh ligands, normally represses the Hh pathway by inhibiting the activity of a key signaling effector called SMO. Consequently, loss of PTCH function in BCCs leads to sustained activation of Hh signaling, which plays a central role in the development and maintenance of these tumors. During hair follicle morphogenesis, precisely regulated expression of Hh ligand, which antagonizes the inhibitory effects of PTCH on SMO, leads to spatially and temporally constrained Hh signaling that is required for proliferation of hair follicle epithelium. To explore the possible functions of Hh signaling in the postnatal hair cycle, we have generated transgenic mice with either epithelium-specific inhibition, or activation, of the Hh pathway. Inhibition of Hh signaling blocks proliferation of hair follicle epithelium during spontaneous and depilation-induced anagen, pointing to an important role for Hh signaling in postnatal growth of the hair follicle. On the other hand, sustained, low-level activation of Hh signaling in the follicle outer root sheath leads to a striking impairment in apoptotic regression of this cellular compartment, coupled with its sustained proliferation, during catagen. These data underscore the importance of Hh signaling in driving proliferation of follicle epithelium during anagen, and suggest that shut-down of Hh signaling is required for programmed regression of follicle epithelium during catagen.

**Session 7A – Non-Invasive and Invasive Hair Techniques for Quantifying and Visualizing Hair Growth**

**S-7A-a**

**Hair Metrix Update**

**Speaker:** Doug Canfield  
**Canfield Imaging Systems, Fairfield, NJ, USA**

[Abstract/summary not available at time of printing]

**S-7A-b**

**GCP-Validation of TrichoScan**

**Speaker:** Rolf Hoffman, MD  
**Freiburg University, Freiburg, Germany**

TrichoScan was validated by the comparative assessment of TrichoScan analysis versus conventional manual visual hair count evaluation. Two validation studies have been performed. The first was conducted in house by the manufacturer, Tricholog GmbH, Freiburg, Germany, and the second under GCP-rules conducted externally by Bioskin GmbH, Hamburg, Germany; a licensed CRO. Digital dermatoscopical images for TrichoScan software analysis were taken from 10 subjects with androgenetic alopecia, with a tattoo location mark present in the measurement area. The measurement area on the vertex of the scalp was shaved on day 1 of the study and 48-hours later the hair was dyed black. All images were analysed by TrichoScan and by three evaluators who manually counted...
hair parameters in every image three times. All images were analysed randomly in a result-blinded fashion. In total, 27,245 hairs were analyzed by hand in the in house validation study and more than 300,000 in the GCP-validation protocol by Bioskin (study 251109BS). The Pearson correlation coefficient revealed a very good correlation of TrichoScan and visual hair counts. Both the in house and the GCP-conforming study.

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<tr>
<td>Cumulative Hair Length</td>
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In addition, data variability has been calculated for TrichoScan and manual evaluation.

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<tr>
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<th>Tricholog Manual</th>
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The margin of error and consequent data variability from manually evaluated images would necessitate a larger study sample size to overcome the effect of the variability in collected data on the statistical significance of the results. As results are highly reproducible with TrichoScan, the smaller margin of operator error and the consistency in the collected data allow statistically significant results to be obtained from studies with a smaller sample size which is pivotal in clinical trials.

Conflict of interest: The author is the inventor and distributor of TrichoScan.

S-7A-c
Dermoscopy of Hair (video)
Speaker: Antonella Tosti, MD
University of Bologna, Bologna, Italy

Video dermoscopy is useful for close examination of the scalp of the follicular ostia and the hair shafts. Magnification ranges from 20x to 80x.

- **Scalp scaling and dandruff**: dermoscopy permits to distinguish psoriasis and sebopsoriasis from seborrheic dermatitis. In psoriasis and sebopsoriasis dermoscopy shows tightly coiled capillary loops which correspond to the tortuous capillaries in the dermal papilla.
- **Patchy alopecia**: alopecia areata can be distinguished from other causes of patchy alopecia though dermoscopy, which shows numerous monomorphous round or polycyclic yellow dots that may centrally have a vellus or intermediate hair. These dots disappear with hair regrowth. The yellow dot pattern is also observed in alopecia areata incognita and represent in our hands the only non-invasive method to diagnose this condition.
- **Androgenetic alopecia**: more than 20% variability in the hair shaft diameter is typical of androgenetic alopecia and diagnostic in the early phases. The presence of hair diameter diversity permits of distinguishing early androgenetic alopecia from chronic telogen effluvium. Peripilar signs, which appears as brown halos around the follicular ostia are a sign of perifollicular inflammation which is often associated with androgenetic alopecia.
- **Inherited and acquired hair shaft disorders**: the hair shaft abnormalities are easily recognized in vivo at high magnification.
- **Scarring alopecia**: in lichen planopilaris and discoid lupus erythematosus, dermoscopy shows scalp atrophy due to loss of follicular ostia and keratotyc plugs around the remaining hairs. In folliculitis decalvans tufted folliculitis are often evident.
- **Nits**: dermoscopy permits to distinguish empty from viable nits.

S-7C
Stress and Hair

S-7C
Overview
Speaker: Ralf Paus, MD
University of Lübeck, Lübeck, Germany

[Abstract/summary not available at time of printing]
Invited Speaker Presentations

Saturday, June 16, 2007

Session 8
Hair Pigmentation

S-8-A
Hair Pigmentation
Speaker: Greg Barsh
Stanford University, Stanford, CA, USA

[Abstract/summary not available at time of printing]

S-8-B
Mechanisms of Melanocyte Stem Cell Maintenance and Hair Graying
Speaker: Emi K. Nishimura, MD, PhD
Kanazawa University Cancer Research Institute, Kanazawa, Ishikawa, Japan

Hair graying is the most obvious sign of aging in man, yet its mechanism is largely unknown. Qualitative and quantitative changes in stem/progenitor cells have been implicated in physiological aging. Melanocytes may be unique in that the oxidative chemistry of melanin biosynthesis can be cytotoxic. This led to the suggestion that differentiated, pigmented melanocytes (rather than their unpigmented progenitors) are specifically targeted in hair graying. The recent discovery of unpigmented melanocyte stem cells (Nishimura EK et al. Nature 2002), distinctly located within the hair follicle, creates an opportunity to determine whether the process of hair graying arises specifically from changes in differentiated melanocytes or the stem-cell compartment which provides them. Here we utilize melanocyte-tagged transgenic mice and aging human hair follicles to demonstrate that hair graying is caused by defective self-maintenance of melanocyte stem-cells. This process is accelerated dramatically with Bcl2-deficiency, which causes selective apoptosis of melanocyte stem-cells within the niche at their entry into the dormant state, but not of differentiated melanocytes. Furthermore, physiologic aging of melanocyte stem-cells was associated with ectopic pigmentation or differentiation within the niche, a process accelerated by mutation of the melanocyte master transcriptional regulator Mitf. Our recent studies provide more insights into the mechanisms of stem cell dormancy and roles of the niche microenvironment for stem cell maintenance.

Session 9
Chemotherapy-Induced Hair Loss

S-9
Molecular Mechanisms of Chemotherapy-Induced Hair Loss: Global Changes in Expression of Apoptotic and Non-Apoptotic Genes During the Response of Human Hair Follicles to Doxorubicin
Speaker: Vladimir Botchkarev, MD, PhD
Boston University School of Medicine, Boston, MA, USA

Authors: T.Y. Sharova1, A.A. Sharov1, R. Atoyan1
A.N. Mardaryev1, V.A. Botchkarev1,2
Laboratory of Skin Development, Regeneration and Carcinogenesis,
1 Dept Dermatology, Boston University School of Medicine, Boston, MA, USA,
2 Medical Biosciences, School of Life Sciences, University of Bradford, UK

Chemotherapy induces DNA damage in rapidly proliferating hair follicle keratinocytes followed by initiation of apoptosis via recruitment of the p53-dependent and independent pathways. To analyze mechanisms involved in initiation of the hair follicle response to chemotherapy, late anagen hair follicles isolated from scalp of healthy individuals were cultured ex vivo with doxorubicin (30 min) and were harvested three hours after treatment. Hair matrix keratinocytes were obtained from the control and doxorubicin-treated hair follicles using laser capture microdissection system (Arcturus), and global microarray analysis was performed using Agilent Whole Human Genome Array. Microarray data validated by the real-time PCR revealed that about 1300 genes show 2-fold and higher differences in expression in doxorubicin-treated hair follicles compared to the control. Vast majority of these genes encoded molecules that are involved in cell adhesion/extracellular matrix remodeling, cytoskeleton/motility, cell signaling/transcription and cell metabolism. Interestingly, doxorubicin-treated hair follicles showed strong increase in the expression of genes encoding keratin-associated proteins, while expression of the hair keratin genes decreased compared to control. Among the genes involved in apoptosis/cell cycle regulation, substantial changes in expression were seen in those involved in p53/Fas-dependent cell death (P53AIP1, FAS, CFLAR), TNF signaling (TNFSF10), as well as in genes encoding cyclin-dependent kinase inhibitors (p21, p57). Thus, these data suggest that the response of hair follicle keratinocytes to chemotherapy is more complex than previously appreciated and include involvement of a large number of genes.

32
whose functions are not directly associated with apoptosis. These data also suggest that p53/Fas and TNF signaling are involved in the initiation of the hair follicle response to chemotherapy and that pharmacological modulation of these pathways may be important for prevention of chemotherapy-induced hair loss.

**Session 12**

**Hair Treatments: What’s on the Horizon**

**S-12-A**

**Laser Hair Treatments**

*Speaker: R. Rox Anderson, MD*

*Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, USA*

Hair removal with selective photothermolysis by targeting follicular melanin, is very popular and clinically useful. Three near infrared laser systems and various xenon flashlamps are used in clinical practice, delivered in combination with skin cooling. The “contest” between follicular and epidermal melanin is such that dark skin is best treated with longer wavelength, longer pulses at lower fluence. While dark coarse hair can generally be permanently removed or reduced regardless of skin type, it is challenging to remove fine and/or light-colored hair. Repeated low-fluence laser treatments can painlessly remove pigmented hair; FDA recently cleared the first laser for home use. What other new laser treatments related to hair follicles will emerge? Optically-stimulated hair growth may occur by at least 3 different mechanisms. Paradoxical stimulation of facial hair by lasers or flashlamps intended for hair removal is seen infrequently in women. Hypertrichosis also occurs after repeated porphyrin-photosensitized exposures, e.g. in porphyria. Topical aminolevulinic acid photosensitizes the entire human hair follicle except arrector pili muscles (root sheath layers, bulge, matrix, papilla and sebaceous gland). Already used widely for acne therapy, photodynamic therapy deserves more study for its effects on human hair follicles. Low-level red and near infrared light may conceivably stimulate hair growth through activation of cytochrome C oxidase, mitochondrial signaling or other pathways. Despite some clinical evidence, optical stimulation of hair growth has not yet been carefully studied. Potentially, light could also be used to affect other changes in hair such as pigmentation, color or curliness.

**S-12-B**

**Nanoparticle-based Targeting of Skin Antigen-Presenting Cells via Hair Follicles**

*Speaker: Annika Vogt*

*Clinical Research Center for Hair and Skin Physiology, Berlin, Germany*

*Authors: Annika Vogt1, Brice Mahe2, Wolfram Sterry1, Behazine Combadiere2, Ulrike Blume-Peytavi1*

1Clinical Research Center for Hair and Skin Physiology, Department of Dermatology and Allergy, Charite – Universitaetsmedizin Berlin, Berlin, Germany;
2Laboratoire d’Immunologie Cellulaire et Tissulaire, Hopital Pitié Salpêtrière, INSERM U543, Paris, France

Drug delivery systems, which target active compounds to the hair follicle, may result in a better penetration and a higher efficiency of hair and skin therapy. Recent studies performed by our group suggest, that nanoparticles in the size range of 40 nm may be used to transcutaneously deliver active vaccine compounds, via the hair follicle, into cutaneous antigen-specific cells. To further investigate the applicability of transcutaneously applied nanoparticles as vaccine carriers, we investigated the penetration and the migratory profile of 40 nm nanoparticles through the skin and to secondary lymphoid organs of C57BL6 mice using in vivo confocal microscopy. We found that 40 nm nanoparticles penetrated deeply into open hair follicles of tape-stripped murine skin. Within 24 hrs diffusion into the perifollicular tissue occurred, and, concomitantly, nanoparticle-positive cells could be identified in proximal draining lymph nodes, mesenteric lymph nodes and the spleen. Transcutaneous application of immunogenic compounds such as DNA plasmids encoding for ovalbumin (OVA) or OVA itself induced proliferation of OVA-specific CD8 T cells. Similarly, transcutaneously applied human influenza vaccine elicited antigen-specific T cells assessed by IFNγ ELISPOT. Our results further strengthen our concept transcutaneous targeting of cutaneous antigen-presenting cells. Further studies using functional particle-bound antigens will help to validate this route of immunisation.

**S-12-C**

**Role of Hair Follicles of Transcutaneous Drug Delivery**

*Speaker: Nina Otberg*

*University of British Columbia, Vancouver, BC, Canada*

*Authors: Nina Otberg, Alexa Teichmann, Heike Richter, Sabine Schanzer, Wolfram Sterry, Juergen Lademann, University of British Columbia, Vancouver, BC, Canada*

The skin with its appendages is the largest organ of the human body. It is our shield against the environment and is necessary for the maintenance of homeostasis. Hypotheses
Concerning the penetration of substances into the skin have assumed diffusion through the lipid domains of the outermost layer of the skin, the stratum corneum. It was believed that hair follicles play a subordinate role in percutaneous penetration processes, although it was presumed that they represent a weakness in the shield. In the past, the investigation of follicular penetration addressed mostly methodical and technical problems. We present different in vivo and in vitro methods for the measurement of the follicular reservoir function and for the determination of transfollicular absorption. We show that hair follicles can form a relevant reservoir for topically applied substances and can act as shunt routes through the skin.

Session 13A
Shape of Hair/Hair Shaft Abnormalities

S-13A-a
Genes in Distinct Types of Murine Hair Follicles: Involvement of BMP Signaling in the Controlling of Hair Thickness and Shape
Speaker: Vladimir Botchkarev, MD, PhD
Boston University School of Medicine, Boston, MA, USA
Authors: A.A. Sharov1, T.Y. Sharova1, A.N. Mardaryev2, R. Atoyan1, and V.A. Botchkarev1,2
Laboratory of Skin Development, Regeneration and Carcinogenesis, 1Department of Dermatology, Boston University School of Medicine, 2Medical Biosciences, School of Life Sciences, University of Bradford

Skin morphogenesis results in the development of the hair follicles (HFs) that generate hairs, whose phenotype (length, thickness, shape and color) varies substantially between distinct anatomical sites of the mammalian body. In murine dorsal skin, HFs are grouped into four principal types (guard, awl, auchene and zig-zag) each characterized by distinct size of the hair bulb and phenotype of the hairs generated. By using laser capture microdissection and global microarray analyses, we show that hair matrix cells of different hair follicle types are characterized by distinct expression profiles of adhesion/extracellular matrix molecules, cytoskeleton/cell motility markers, molecules involved in the control of cell differentiation, metabolism, signaling and transcription. We also show that bone morphogenetic protein (BMP) signaling is involved in the regulation of hair follicle size and hair shape. Transgenic (TG) mice overexpressing the BMP antagonist noggin (promoter: K5) are characterized by the replacement of zig-zag and auchene hairs by awl-like hairs and by marked increase in size of anagen hair follicles (HFs), compared to the age-matched wild-type (WT) controls. Markedly enlarged anagen HFs of TG mice show increased proliferation in the matrix and increased number of hair cortex and medulla cells compared to wild-type HFs associated with a strong decrease in expression of cyclin-dependent kinase inhibitor p27^KIP1 and increased expression of selected cyclins in TG versus WT mice. These data suggest that BMP signaling plays an important role in controlling hair shaft thickness and shape via modulating cell proliferation and expression of cell-cycle associated genes in hair matrix keratinocytes.

S-13A-b
Characterization of Human Hair Shape: From Hair Bulb to Hair Fiber
Speaker: Bruno Bernard, PhD
L’OREAL Recherche, Clichy, France.
Authors: S. Thibaut, S. Malgouries and B. A. Bernard, L’OREAL Recherche, Clichy, France.

Objective: We investigated curly hair morphology and the formation of the hair shaft.

Approach: A comparative study was carried out on a set of human hair whose shapes ranged from straight to tightly curly. Immunohistology and in vitro culture were used to study the specific features of curly hair bulbs. Hair macrofibril organization was investigated by transmission electron microscopy experiments.

Results: We observed that the curly hair bulb exhibited a retro-curvature. When these follicles were micro-dissected and in vitro cultured, the curvature was maintained in the newly formed hair shaft. At the cellular level, the direct comparison of straight hair and curly hair highlighted an intrinsic asymmetry of the proliferative compartment that clearly extended above the Aubé line on the convex side of the curvature. This phenomenon led to a delayed differentiation of the inner root sheath and the outer root sheath. The hair cortex itself was elliptical and asymmetric. hHa8 keratin was accumulated in the concave side of the curvature, whereas in straight hair, positive cortical cells were homogeneously distributed around a circular fiber. This asymmetric arrangement of intermediate filaments in the precortex area of curly hairs could possibly reflect some ortho- versus para cortex segregation. In addition, alpha-smooth muscle actin tension marker was synthesized at the very beginning of ORS differentiation, underlining a mechanical stress in the curvature.

Conclusion: The curly shape of the hair seemed to be a consequence of the asymmetric differentiation of the hair bulb in addition to specific signals from the outer root sheath.
Invited Speaker Presentations

Saturday, June 16, 2007

**Session 13B**
**Nutrition and Hair Growth**

**S-13B-a**
**Nutrition and Hair Growth**
**Speaker:** Ralph M. Trüeb, MD
*University Hospital of Zurich, Zurich, Switzerland*

The quantity and quality of hair growth are closely associated with the nutritional state of an individual. Normal supply, uptake, and transport of proteins, calories, trace elements, and vitamins are of fundamental importance in tissues with a high biosynthetic activity such as the hair follicle. It appears that on a typical Western diet, the hair follicle should have no problem in producing an appropriate hair shaft. Nevertheless, in instances of protein and calorie malnutrition, deficiency of essential amino acids, of trace elements, and of vitamins, hair growth and pigmentation may be impaired. The nutritional defect may be environmental or due to a hereditary defect in absorption or metabolism. Response to substitution therapy is usually good. Since an important commercial interest lies in the nutritional value of various vitamin and amino acid supplements, a question that arises is whether increasing the content of an already adequate diet with specific amino acids, vitamins and/or trace elements may further promote hair growth, particularly in the aging hair follicle, where a physiological deficit is hypothesized.

**S-13B-b**
**Supplements, Iron and Hair Growth**
**Speaker:** Wilma Bergfeld
*The Cleveland Clinic Foundation, Cleveland, OH, USA*

Iron deficiency is the world's most common nutritional deficiency. In young women the most common cause is excessive menses and diet while in young men the most common is diet. In the pre-menopausal and menopausal women the most common cause is menorrhagia. Hemoglobin concentration is screen for iron deficiency while ferritin is identifies iron storage deficiency. Elevated ferritin can be attributed to infectious, inflammatory, neoplastic conditions or from excessive iron replacement. Additional laboratory test can include erythrocyte zinc protoporphyrin concentration, transferring concentration, serum iron concentration and transferring saturation. If the cause of iron deficient is not attributed to diet or excessive menstruation, other causes should be investigated.

Several published studies suggest that suggest a relationship between iron deficiency and hair loss. Hair loss secondary to iron storage deficiency and, iron deficiency has been observed telogen effluvium, diffuse pattern hair loss, and alopecia areata. In these disorders, an identification of low hemoglobin and ferritin, and treatment resulted in clinical improvement. Therapy consists of iron supplements; iron containing foods and treatment of the underlying cause. Laboratory monitoring is initially recommended every 8 weeks. In vegetarians, vega vegetarians or in patients with chronic menorrhagia, chronic iron supplement is necessary. The Institute of Medicine recommends: the upper limit of iron intake for men and pregnant and non pregnant women of 18 years of age of older is 45 mg/day. The major side effect is iron overload which can result in tissue damage and fibrosis and exacerbation of hereditary hemochromatosis.

**S-13B-c**
**Female Pattern Hair Loss and Iron – My View**
**Speaker:** Hugh Rushton
*School of Pharmacy & Biomedical Sciences, Portsmouth, United Kingdom*

The unit area trichogram was developed in late 70's. With the new ability to identify the three fundamental hair variables involved in hair loss, and the emerging understanding of the role of anti-androgens in hair biology; it seemed only a matter of time before we could help women with non-scarring hair loss. So what has happened over the last 30 years?

The fundamental variables have not changed nor has the ability to evaluate them. However, we now have to deal with lag phases, exogen release, nutritional influences, and non-responders to therapies once thought to be the answer to treating female pattern hair loss. In addition, we now deal with patients presenting with new syndromes:- IRIOTI (I Read It On The Internet), SAS (Seen All Specialists), TAT (Tried All Treatments) and SIR (Select Information Retention). Further, recent concern about hormone replacement therapy (HRT) and hormonal manipulation in premenopausal women has not helped.

Female pattern hair loss presenting at any age is neither serious nor life threatening but for those who suffer it can adversely affect their quality of life. The desire to find treatment modalities has been painfully slow and recent pressures on the medical and pharmaceutical professions, together with the restrictions on cosmetic companies to develop effective ‘cosmo-pharmaceuticals,’ has not helped. So where does this leave us for the future?

Perhaps it is time for me to retire!
O-01-1

Stem Cells of Human Hair Follicles Can Differentiate Into Neurons: Region-Specific Multipotency of Human Hair Follicle Stem Cells

Amoh, Yasuyuki;1 Hamada, Yuko;1 Niiyama, Shiro;1 Tamauchi, Hidekazu;2 Kawahara, Katsumasa;1 Satoh, Yuichi;3 Hoffman, Robert M.;4 Katsuoka, Kensei;1
1. Department of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan; 2. Department of Microbiology, Kitasato University School of Medicine, Sagamihara, Japan; 3. Department of Molecular Diagnostics, Kitasato University School of Allied Health Sciences, Sagamihara, Japan; 4. AntiCancer, Inc., San Diego, CA, USA

Hair follicle stem cells in the hair follicle bulge area of mice are nestin- and CD34-positive, and keratin 15 (K15)-negative. The mouse hair follicle stem cells are multipotent and can differentiate into neurons, glial cells, keratinocytes, smooth muscle cells, and melanocytes (Proc. Natl. Acad. Sci. USA 102, 5530-5534, 2005). Recently, pluripotent stem cells have been identified in human hair follicles (Am. J. Path. 168, 1879-1888, 2006). The stem cells in hair follicles of human scalp express nestin and embryonic-stem-cell transcription factors Nanog and Oct4. The human hair follicle stem cells also can differentiate into neurons, smooth muscle cells, and melanocytes.

In the present study, we observed that the plucked anagen hair follicles of the human scalp contained the K15-positive hair follicle cells. The plucked hair follicles were cut into upper, middle, and lower parts, and suspended in DMEM-F12 containing B-27 supplemented with basic FGF every two days. After 10 days, only upper part of the hair follicle formed the cell colonies, and differentiated into the K15-expressing keratinocytes. These results suggest that K15-positive cells in the plucked hair follicles contain the keratinocyte progenitor cells. Moreover, some cells from the upper part of the hair follicles also differentiated into neurons. Current experiments will determine optimal conditions for the human hair follicle stem cells to differentiate into other cell types.

O-01-2

Adult Stem Cell Compartment Changes in Androgenetic Alopecia Demonstrate Maintenance of Progenitor Stem Cells With Loss of Descendant CD200 High A6 Integrin High Expressing Cells

Garza, Luis A.; Lee, Michelle; Liu, Yaping; David, Stanton; Lee, Carrasco; Tobias, John; Costarelli, George, University of Pennsylvania, Philadelphia, PA, USA

The status of adult stem cell compartments in tissue specific disease has not been thoroughly addressed. We tested the hypothesis that hair follicle stem cells might be depleted in androgenetic alopecia (AGA), which is characterized by drastic miniaturization of the hair follicle. To compare hair follicle stem cell numbers between paired haired and bald scalp samples from the same individuals, we used flow cytometry to quantitate cell cycle, cell size, and expression of CYTOKERATIN 15 (KRT15), FOLLISTATIN (FST), CD200 and alpha-6 integrin. We found a gradient of stem cell characteristics, as defined by a high degree of KRT15 and FST expression, cellular quiescence and small cell size. This gradient is not grossly altered between haired and bald scalp, and stem cells are maintained in bald scalp. However, a specific CD200 high alpha-6-integrin high population, which has characteristics of early stem cell progeny, is lost in bald scalp. Consistent with the loss of the immunosuppressive CD200 protein, array based expression profiling demonstrates significant increases in inflammation associated genes in androgenetic alopecia. Previous reports of CD200 loss leading to alopecia in mouse models suggest that AGA may be exacerbated or caused by CD200 downregulation in the human hair follicle stem cell compartment.

O-01-3

Adult Hair Follicle Dermal Papillae Induce Hair and Skin Differentiation From Adult Corneal Epithelium

Richardson, Gavin D.;1 Waters, James;2 Fern, Amy;2 Dhouailly, Danielle;3 Taghizadeh, Reika;2 Jahoda, Colin;2
1. Durham University, Durham, UK; 2. Department of Biological Sciences, University of Durham, Durham, UK; 3. Biologie de la Differentiation Epitheliale, Institut Albert Bonniot, Grenoble, France

It has been shown previously that both microdissected DP and cultured adult DP cell aggregates are capable of inducing new follicles from hairless skin. It has also been demonstrated that embryonic dermis is capable of inducing hair follicles and skin from central corneal epithelium. Using microdissection and tissue combination techniques we investigated whether DP were capable of inducing new hair follicle structures in other epithelia including the cornea and oral mucosa.

Rabbit corneal epithelium was separated from the underlying stroma using EDTA, and combined with dermal papillae isolated from rat or mouse vibrissae follicles placed on top of supporting rat footpad dermis. Oral mucosa from rat was treated enzymatically to separate the epithelia and mesenchyme before dissected DPs from rat or mouse were inserted between the two layers. The tissue combinations were inserted beneath the renal capsule of athymic mice for up to one month before cryostat sectioning and immunofluorescent analysis.
We have found that microdissected DP are capable of inducing new hair follicle structures and local epidermal differentiation in cornea, as characterised by expression of keratins from the hair (AE13), basal epithelium (K14). Interestingly we also observed epidermal differentiation, as indicated by K10 expression. These events emphasize the potency of DP signalling, and currently we are investigating the early events involved in new follicle induction. The findings also illustrate the capacity for the lineage committed transit-amplifying corneal cells of the central cornea to dedifferentiate into more stem cell-like progenitors capable of then becoming different epithelial cell types.

O-01-4
Bone Morphogenetic Protein Signaling Is Required For Hair Induction By Dermal Papilla Cells

Rendl, Michael; Fuchs, Elaine; Laboratory of Mammalian Cell Biology and Development, Howard Hughes Medical Institute, Rockefeller University, New York, NY USA

New hair follicle formation in embryonic and adult skin is initiated when specialized mesenchymal dermal papilla (DP) cells send cues to multipotent epithelial stem cells. Later during active hair growth, DP cells form a niche with neighboring epithelial cells to orchestrate the complex program of hair shaft differentiation. When taken outside the niche into culture, however, DP cells lose their hair follicle inducing properties. To explore what lies at the heart of these processes, we recently developed methods to isolate and characterize the molecular identity of the DP and its niche as cell-type specific gene signatures. Using growth factors and other signaling molecules from these signatures, we tested whether cultured DP cells could be manipulated to maintain their molecular identity and hair follicle inductivity. Of all applied factors, only bone morphogenetic proteins (Bmps) maintained molecular DP signature features in vitro and moderately preserved hair induction in vivo. Conversely, Bmpr receptor ablation specifically in DP cells in a novel in vitro/vivo hybrid knockout assay strongly reduced hair formation alongside with a corresponding loss of molecular DP signature features. These results put the Bmp pathway squarely at the center of DPs function of hair induction. However, since Bmps are not sufficient to fully maintain hair inductivity, we propose that combinatorial application with other factors will prove useful to unleash their full potential in the future.

O-03-1
Regeneration of Human-Mouse Chimeric Follicles in a Hybrid Patch Assay

Zheng, Ying; Boucher, Marylene; Homan, Ying; Chen, Wei; Parimoo, Satish; Stenn, Kurt; Aderans Research Institute, Philadelphia, PA, USA

Previously we have reported a highly efficient and reliable method to regenerate hair follicles in vivo from dissociated mouse cells in a hair regeneration system, the “Patch assay”. We adapted this method to examine if human and mouse cells could generate human-mouse chimeric follicles in the nu/nu mice. Cultured human adult dermal or epidermal cells from scalp were combined with C57/Black mouse neonatal epidermal or dermal cells and injected intradermally into immunoincompetent (nu/nu) mouse skin. Chimeric follicles formed in 3-4 weeks. A histological time course study revealed that the human dermal/mouse epidermal or the human epidermal/mouse dermal cells in the patch assays formed chimeric follicles in a manner similar to that seen in mouse-mouse cells. In this system epidermal cells form aggregates at 2-3 days after injection; at this stage dermal cells surrounding these aggregates show no obvious dermal condensate structures. The epidermal aggregates then form cysts by an apoptotic mechanism at the center of the aggregates. The cysts fuse with each other to form an epithelial platform with placode-like structures. Dermal condensates, suggestive of follicular papilla formation occur at around 10 to 15 days with down-growth of epithelial cells, and mature follicles form in 17 to 22 days. Staining with human or mouse-specific centromere probes showed a dynamic interaction between human and mouse cells. This hybrid patch assay is an effective tool to study trichogenicity of human cells and mesenchymal-epithelial interaction during hair follicle formation.

O-03-2
Methods of Follicular Cell Implantation for Hair Multiplication

Teumer, Jeff; Qiao, Jizeng; Intercytex, Woburn, MA, USA

Follicular cell implantation (FCI) is a cell therapy for hair multiplication currently under study in human clinical trials. The challenge is to implant hair inductive cells in a manner that achieves efficient hair restoration and good value to the patient. The therapy is based upon the hair inductive capacity of cultured adult dermal papilla cells (DPC). We have previous demonstrated the ability to achieve significant expansion of hair inductive human DPC cultured under Good Manufacturing Practice (GMP) conditions. Hair formation requires keratinocytes in addition to inductive DPC, and keratinocytes can originate from a variety of sources such as truncated follicles, glabrous...
epidermis, cultured follicular keratinocytes and cultured neonatal keratinocytes. These possible sources have been demonstrated in various experimental systems. Each keratinocyte source, when combined with cultured DPC, provides an alternative strategy for FCI. For example, if interfollicular epidermis were the source of keratinocytes in new follicle formation, keratinocytes would originate from the implant site and a formulation of DPC alone would be implanted, while if cultured keratinocytes were the source they would be co-implanted in a combination formulation of DPC with keratinocytes. We show hair formation using several formulations for follicular cell implantation that range from single cell suspensions to transplantable immature follicles formed in vitro. The implications of the various formulations will be discussed in the context of the clinical development of FCI for hair multiplication.

O-03-3
Expression of TGF Beta2 in Cultured Human Dermal Papilla Cells and Its Ability of Induction of Tissue Engineered Hair Follicles

Inoue, Keita; Sato, Takahiro; Yamauchi, Yuji; Murase, Shoko; Yoshimura, Kotaro
1. University of Tokyo, Tokyo, Japan; 2. Biomaster, Inc., Yokohama, Japan

Introduction: TGFb2 is known as a potent catagen inducer of human hair follicles in adult scalp skin, whereas it is known to be a sufficient inducer of murine hair morphogenesis in the developing skin.

Materials and Methods: We performed a microarray analysis and RT-PCR comparing human dermal papilla cells and dermal fibroblasts both obtained from adult scalp skin, in order to elucidate the genetic factors responsible for hair induction in tissue engineered hair follicles.

Results: In the microarray analysis we found that TGF b2 gene was significantly upregulated compared to dermal fibroblasts and that it was one of the candidate genes involving in hair regeneration. RT-PCR analysis revealed significant and sustained upregulation of TGF b2 mRNA in cultured dermal papilla cells compared to dermal fibroblasts up to 8th passage when they were cultured in DMEM. Furthermore, we found soluble factors secreted by keratinocytes significantly upregulated TGF b2 mRNA.

Discussion: Various growth factors secreted by cultured keratinocytes have proliferative effects on human dermal papilla cells in dose dependent manner, and promotes hair follicle induction in rat sole transplantation models. TGF b2 gene seems to be upregulated in dermal papilla cells via transcriptional pathway stimulated within epithelial-mesenchymal interaction.

Conclusion: TGF b2 gene was upregulated in cultured human dermal papilla cells, and further upregulated by soluble factors secreted by keratinocytes. TGF b2 may be one of possible inducers of tissue engineered human hair follicles.

O-03-4
In Vitro Generation of Human Hair Follicle Bud Oriented Cellular Mass Composed of Dermal Papilla Cells and Keratinocytes

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On the purpose of making human hair buds in vitro, previously we reported a new experimental technique about an In vitro generation of human somatic cellular mass (CM) composed of dermal papilla cells (DPs) and human epidermal keratinocytes using the hanging-drop culture system. In the above, we showed that an application of GSK-3 beta inhibitor 9 (BIO) enhanced the development and growth of CMs. In this study, we observed that CMs were positive for an alkaline phosphatase activity and Ber-EP4 immunoreactivity. The region of epithelial cells showed the immunoreactivity against CD34 antigen and cleaved Notch-1. Especially the CMs composed of BIO-treated DPs were clearly positive against CD34 antigen and an alkaline phosphatase activity compared with controls. Moreover a quantitative PCR result showed that CMs expressed Wnt10b, 5a and other Wnt related genes. The expression rates of these genes in CMs were well synchronized and partially similar to in vivo developing hair follicles. These data suggested that the BIO activated CMs might indicate the similarities to the follicular development and/or the stage of anagen induction with the sequential expression of Wnts and Wnts related genes.

O-03-5
The Hair-Inducing Clonal Cell Lines From Dermal Papilla and Dermal Sheath Cells of Mouse Vibrissa Follicles

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Dermal papilla (DP) and dermal sheath (DS) cells have the ability to induce de novo hair follicle formation and DP cells are thought to be derived from DS cells. Recently, some researchers reported that DP is a reservoir of adult mesenchymal stem cells, and that DS cells also have multipotent abilities as well as DP cells. Previous study revealed the clonal growth of DP or DS cells after explant culture. In this study, we isolated single DP cells...
and DS cells from mouse vibrissal follicles directly, and succeeded to produce clonal cell lines using feeder cells. In our culture method including FGF2 (presented at EHRS 2006), most of the clones stopped proliferating within several passages, but a few lines continued to grow over 10 passages. At passage two most of the clones induced de novo hair follicle formation when they were combined with adult epithelium and implanted into athymic mice. Each clonal cell lines which retained hair follicle inducing abilities showed difference in cell shape and expressed different genes. Our results suggest that hair-inducing cells may be heterogeneous. We are currently examining the multipotency of each clone in vitro.

**O-03-6**

**Large-Scale Production of Dermal Papilla Microtissues Via Facilitated Self-Assembling: Implications For Hair Follicle Engineering and Dermal Papilla Physiology**

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Neogenesis of hair follicle in adult life has been demonstrated by transplanting cultured dermal papilla cells in dense aggregates or microtissues, an intercellular organization similar to the physiological condition of dermal papilla cells. In tissue engineering for hair follicle reconstruction, there have been no efficient methods to produce dermal papilla microtissues on a large scale. Further, there is currently lack of an in vitro model that allows the examination of the intrinsic aggregative behavior of dermal papilla cells to be achieved. In this work, we demonstrate an in vitro system that facilitates the self-assembly of dermal papilla cells into microtissues. We show that EVAL (poly (ethylene-co-vinyl-alcohol)) surface is able to support the cell proliferation of dermal papilla cells. Seeded above a critical density, dermal papilla cells spontaneously grow into scattered dense multicellular microtissues on EVAL surface after 3 days in culture. The cells are viable in the microtissues and able to grow when they are reseeded. The differentiation markers of dermal papilla cells are preserved in the microtissues. Dynamically, the formation of dermal papilla microtissues can be divided into 3 steps: active cell migration, intercellular collision and multicellular aggregation into microtissues. Interestingly, the formed microtissues are able to move collectively, a unique behavior similar to that of dermal papilla cells during hair follicle cycling in vivo. This system allows future investigation of the self-assembling behavior of dermal papilla cells. With further development, it can also help to produce dermal papilla microtissues of tailored dimensions on a large scale for clinical and pharmaceutical applications.

**O-04A-1**

**Re-evaluation of Natural Hairline Patterns and Recession Patterns of the Frontal and Midscalp Zones in Men**

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It has long been known by hair restoration surgeons that the creation of irregularities at the frontal hairline is desirable in trying to obtain an undetectable result. This has often led to the creation of irregular, but unnatural designs, that don’t achieve their goal. One of the irregularities in a natural hairline is the presence of projections of the hairline, particularly near the central portion. 45% of 100 consecutive hairlines evaluated had 1-3 projections of the hairline beyond the natural rounded hairline. These “macro irregularities” are easily seen from a few feet away and have been termed peaks (mounds). The central peak (widow’s peak) is well known to the general public, but less well known is the other 2 potential peaks. Their creation during hair restoration can add to a natural effect.

Smaller “micro irregularities” are also seen along the frontal hairline. They consist primarily of small clusters, gaps, and random single hairs. On casual glance the frontal hairline appears straight but closer inspection reveals the irregularities. Most experienced hair surgeons try to create these micro irregularities in every case in which a frontal hairline is being created.

Another common tool used to simulate a natural hairline is the creation of a “flare” of the frontal hairline just before it blends into the temporal-parietal fringe. Most commonly this flare is simply drawn in a pleasing manner with few apparent guidelines. However, it appears that this flare is not often a natural part of the lateral frontal hair line; but appears to be most commonly related to the frontal hairline recession blending into a retained mid-scalp. With this realization, some parameters can be set for designing a frontal flare. Multiple photos will be used to develop this point.
O-04A-2
Calculation of Donor Hair Density, Strip Size and Transection Rates in Hair Restoration Surgery

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Objectives: Hair restoration surgery is an important treatment option in pattern hair loss. Exact calculation of the donor area and the expected number of transplanted hair follicles is crucial for patient satisfaction and efficient cost calculation.

The aim of this study is to provide data on donor area hair density in hair transplantation before the procedure, using macro-photography and digital imaging software. Hair density will be compared to the number of extracted hair per cm².

Approach: Digital macro-photographs of 20 fold magnification are taken before and after administration of tumescent anesthesia directly before the donor strip harvesting. Hair density will be measured using the digital imaging tool and software Trichoscan(r). The total expected number of follicles in the donor strip is calculated and later correlated to the number of harvested and transplanted hair.

Results: Ten male and female patients were enrolled in the study. Hair density ranged between 110 and 192 terminal hair/cm². After the administration of the local anesthetic the skin stretches and hair density decreased by 1-10%. The number of the harvested and transplanted hairs correlated well with the calculated number. The transection rate was less than 4%.

Conclusion: Trichoscan(r) technique allows a better calculation of the donor strip size and dissection and transection rates. Transection and dissection rate can be calculated more exactly. Decrease in hair density of up to 10% after the administration of the tumescent anesthesia has to be taken into account.

O-04A-3
Hair Transplant in Asians

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Singapore is a multiracial society with different Asian ethnic groups. The ethnic characteristics have advocated the use of micrograft megasessions for the purpose of a more natural looking end result, especially in the Oriental with higher skin/hair color contrast and darker, coarse, straight hairs. But it also has some fundamental limitations. In this presentation, the unique consideration of hair transplantation in Asian will be discussed.

O-04C-1
Autosomal Dominant and Autosomal Recessive Monilethrix – Report of 28 Families

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Introduction: Monilethrix is a structural defect of the hair shaft usually inherited in an autosomal dominant fashion and caused by mutations in the hHb1, hHb3 and hHb6 keratin genes. Autosomal recessive inheritance in this disease has been sporadically reported.

Objective: To find the genetic basis in 28 families with microscopic and clinical findings of monilethrix.

Approach: Physical examination, microscopic hair examination and direct sequencing of the hHb1, hHb3, hHb6 hair keratin genes and DSG4 gene, on DNA extracted from peripheral blood lymphocytes.

Results: In twelve of the 28 families studied, where autosomal dominant inheritance was obvious, we found three mutations in the hair keratin gene hHb6. In two out of the remaining 16 families with no evidence of vertical transmission, two de-novo mutations were found in hHb1 and hHb6. In the 14 Jewish families originating from Iraq, Iran and Morocco no mutations were found in these three hair keratin genes, and therefore we examined nine chromosomal regions known to contain gene clusters encoding skin and hair genes. On chromosome 18q, a common haplotype in the homozygous state was found among all seven Iraqi patients but not in 20 controls (P < 0.0001). Sequencing of the main candidate gene in this region revealed 4 different mutations in desmoglein 4 (DSG4), previously reported to carry mutations in localized autosomal recessive hypotrichosis.

Conclusions: Our findings clarify the basis for autosomal dominant and autosomal recessive monilethrix and have important implications for genetic counseling to monilethrix patients and their families.
O-04C-2
Atrichia With Papular Lesions at Young Age May Be Misdiagnosed as Patchy Alopecia Areata

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Introduction: Atrichia with papular lesion (APL) is a rare autosomal recessive disease, characterized by complete irreversible hair loss during the first months of life. The clinical picture resembles the more common condition, alopecia areata universalis. The presence of alopecia is followed by the appearance of papules on the scalp and the extremities. APL results from mutations in the hairless gene (HR) on chromosome 8p12. At present, no treatment is available.

Objective: To describe an eighteen month-old child with APL, emphasizing her unique clinical features, and to report HR gene analysis.

Approach: Physical examination and direct sequencing of the HR gene on DNA extracted from peripheral blood lymphocytes.

Results: At 18 months, the child presented with patchy alopecia, very similar to patchy alopecia areata, and scattered skin papules located on the scalp and dorsal aspects of the hands. Direct sequencing revealed a recurrent homozygous 2147delC, leading to a frameshift and premature termination codon, 544 base pairs downstream, at exon 12. At present, no treatment is available.

Conclusion: In early childhood, APL may present very similarly to alopecia areata. Since a skin biopsy is very seldom performed in such a young population, clinical misdiagnosis may lead to futile treatments. A genetic test is an important tool for rapid confirmation of the correct diagnosis. In view of the fact that APL may easily be misdiagnosed clinically in this age group, we therefore assume that this condition is far more common than previously estimated.

O-04C-3
Increased Expression of EctodysplasinA1 and Ectodysplasin Receptor Coincides with the Formation of Primary Wool Follicles in Sheep

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Primary wool follicles are initiated in embryonic development at approximately d50 of gestation, as the result of dynamic interactions between the epidermal placode of the ectoderm and the dermal condensate of the underlying mesenchyme. Morphological conservation in formation of skin appendages among mammals allows research into human skin conditions to be completed using sheep as a model organism. X-linked hypohidrotic ectodermal dysplasia (ED) is characterised by abnormal hair and teeth formation and an absence of sweat glands. Mutations within the Ectodysplasin A1 (EdaA1) and its receptor (Edar) have been characterised and established as the cause of ED. Furthermore, it has been shown that interactions between the ectodysplasin pathway and bone morphogenetic proteins drive the follicle patterning process.

Objectives: The aim of this study was to investigate the role of EdaA1 and Edar in initiating primary wool follicles, specifically their role in establishing the primary follicle pattern and involvement in epidermal:mesenchymal interactions.

Approach: A foetal skin series was generated with four skin samples taken at eight time points from day 43 to 68 of gestation. Expression of mRNA encoding EdaA1 and Edar was examined using quantitative PCR and expression data were normalised to GAPDH.

Results: EdaA1 showed a steady increase in expression from d43 to d60, peaking at a 1.6-fold increase (p<0.005). Edar expression demonstrated a 7.5-fold increase from day 43 to d68 (p=0.0001).

Conclusion: These results suggest an increase in expression of mRNA encoding EdaA1 and Edar coinciding with establishment of primary wool follicles and specifically the initiation of the epidermal:mesenchymal interaction required for follicle maturation.

O-05-1
Wnt-Dependent De Novo Hair Follicle Regeneration in Adult Mouse Skin Following Wounding

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The mammalian hair follicle is a complex “miniorgan” thought to form only during development; loss of an adult follicle is considered permanent. Here we show that, after wounding, hair follicles form de novo in genetically normal adult animals. The regenerated hair follicles establish a stem cell population, express known molecular markers of follicle differentiation, produce a hair shaft, and progress through all stages of the hair follicle cycle. Lineage analysis demonstrated that the nascent follicles arise from epithelial cells outside of the hair follicle stem cell niche, suggesting
that epidermal cells surrounding the wound assume a hair follicle stem cell phenotype. Inhibition of Wnt signaling after reepithelialization completely abrogates this wounding induced folliculogenesis, while overexpression of Wnt ligand in the epidermis increases the number of regenerated hair follicles. These remarkable regenerative capabilities of the adult support the notion that wounding induces an embryonic phenotype in skin, and that this provides a window for manipulation of hair follicle neogenesis by Wnts. These findings suggest novel treatments for wounds, hair loss and other degenerative skin disorders.

O-05-2

P-Cadherin Is a p63 Target Gene With a Critical Role in the Developing Limb Bud and Hair Follicle

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P-cadherin is a member of the classical cadherin family that forms the transmembrane core of adherens junctions. Mutations in the P-cadherin gene (CDH3) have been shown to cause two inherited diseases in humans; hypotrichosis with juvenile macular dystrophy (HJMD; OMIM 601553) and ectodermal dysplasia, ectrodactyly, macular dystrophy (EEM syndrome; OMIM 225280). The common features of both diseases are sparse hair and macular dystrophy of the retina, while only EEM syndrome also shows the additional finding of split hand/foot malformation (SHFM). We recently identified four consanguineous Pakistani families with either HJMD or EEM syndrome, and detected pathogenic mutations in the CDH3 gene of all four families. In order to define the role of P-cadherin in hair follicle and limb development, we performed detailed expression studies of P-cadherin in the mouse embryo, and demonstrated the predominant expression of P-cadherin not only in the hair follicle placode, but unexpectedly, also at the apical ectodermal ridge (AER) of the limb buds. Based on the evidence that mutations in the p63 gene also result in hypotrichosis and SHFM (EEC syndrome; OMIM 604292), and that its expression co-localizes to the hair follicle placode and AER along with P-cadherin, we postulated that CDH3 could be a direct transcriptional target gene of p63. To test this hypothesis, we performed promoter assays and chromatin immunoprecipitation, which revealed that p63 directly interacts with two distinct regions in the CDH3 promoter. We conclude that P-cadherin is a newly-defined transcriptional target gene of p63, with a critical role in hair follicle morphogenesis as well as the AER during limb bud outgrowth.

O-05-3

The Wnt Inhibitor, Dickkopf 4, Is Induced By Canonical Wnt Signaling During Ectodermal Appendage Morphogenesis

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Ectodermal appendage morphogenesis requires continuous epithelial-mesenchymal cross-talk during development. Canonical Wnt signaling has been shown to be pivotal during this process and its inhibition leads to the absence of any morphological or molecular signs of appendage formation, including hair follicles (HFs). In the mouse, primary HFs arise in utero starting just before E14.5, when the first morphological signs of a placode are discernible. In this study, our goal was to identify novel factors expressed during primary HF morphogenesis. We performed transcriptional profiling of the developing epidermis at 12 hour intervals between E12.5 and E15.5. One of the significantly differentially expressed genes was the Wnt inhibitor Dickkopf 4, Dkk4. We show that Dkk4 mRNA increases sharply in the dorso-lateral epidermis around E14 and then decreases until E15.5. Using whole mount in situ hybridization, we show that Dkk4 mRNA is localized to the pre-placodes at sites of presumptive epithelial-mesenchymal interactions during appendage morphogenesis, including the dental lamina, mammary gland, eccrine gland, and primary and secondary HFs. In silico analysis, reporter gene assays as well as in vitro transfections of LEF1 and b-catenin show that Dkk4 is a potential downstream target of canonical Wnt signaling. In addition, we demonstrate a direct physical interaction between LEF1/b-catenin complex and the DKK4 promoter using ChIP, showing that Dkk4 is a direct downstream target of this pathway. We propose that Dkk4 acts in a negative feedback loop to attenuate canonical Wnt signaling via a reaction-diffusion mechanism, and may facilitate the non-canonical Wnt planar cell polarity (PCP) pathway that maybe involved in cell movements during appendage morphogenesis.

O-05-4

Molecular Signature of the Follicular and Glandular Types of Epidermal Differentiation: Evidence That BMP Signaling Suppresses Trans-Differentiation of the Foot Pad Epidermis Towards Folliculogenesis

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Objectives: During skin development, epithelium and mesenchyme interact each other and give rise to the formation of a variety of appendages. However, molecular signature and mechanisms underlying formation of the distinct types of skin appendages (hair follicles [HFs], sweat glands [SWG]), remain to be clarified.

Approach: Global gene expression profiles of the epithelial buds of the HFs and SWGs, as well as of hair matrix keratinocytes and SWG epithelium obtained from embryonic and adult mouse foot pads and dorsal skin by laser capture microdissection were performed using the Agilent platform.

Results: Epithelial buds of the HFs and SWGs showed expression of the bud-specific adhesion molecules, signaling/transcription components, as well as appendage-specific markers such as HF-specific keratins (Krt1-c29, Krt2-6g) or SWG-specific ion channels (Clcn3, Bcng-3a). Fully developed SWGs were characterized by strong downregulation of the epidermis-specific genes and by upregulation of SWG-specific genes involved in regulation of the ion exchange/water metabolism, while HFs showed less prominent differences in gene expression versus the epidermis. In addition, SWGs and HFs showed differences in expression of a number of molecules involved in the BMP pathway, while K14-Noggin and Bmpr-Ib knockout mice showed ectopic formation of the HFs in foot pads accompanied by decreased expression of Engrailed 1, a potent repressor of the dorsal phenotype in ventral epidermis.

Conclusion: HFs and SWGs are characterized by markedly different gene expression profiles, which may underlie fundamental differences in their functions. BMP signaling suppresses trans-differentiation of the foot pad epidermis towards folliculogenesis at least in part via stimulating the Engrailed 1 expression.

O-7A-1
e-Hair Analysis Via the IntHairNet Platform

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The authors agreed to launch an electronic platform through which phototrichogram (PTG) technology might become available for a global worldwide network of clinics committed to hair evaluation. Four centers agreed to participate in a collaborative project. PTGs were performed according to a standardized protocol using contrast enhancement and exogen hair collection. Source documents, processed images, computer-assisted-image-analysis (CAIA) and the final PTG data are a two-way exchange process via IntHairNet with a secured access code. Analytical CAIA-PTG data e.g. thickness (20μm≤Ø≤100μm) and growth parameters (anagen, catagen-telogen, exogen) for each individual hair fibre entered the database. From there, hair parameters can be downloaded for communication to the patient or for a specific research project.

Because many hair-skin types (e.g. phototypes) and also socio-cultural differences exist in grooming habits, we wished to establish which parameters remained unaffected by rubbing the scalp daily with “ineffective” lotions. CAIA-PTG was planned in 20 MPHL subjects 3 times at 1 month interval (m0, m1, m2) and m1-m2 data expressed as a % of the m0 values. Statistical analysis showed that growth rate of intermediate hairs (30μm≤Ø≤60μm) and % of growing hair (Ø≥30μm) were the most stable variables (<3% variation). The variation in total hair count, % of growing hair (Ø≥40μm) and telogen counts (Ø ≥20μm) varied between 3 and 5%.

These variables (combined “biological-technological-rubbing” effect) may represent the most robust parameters to study drug related hair growth changes over a short time interval.

In summary, the IntHairNet feasibility study illustrated that high tech, reproducible and accurate measurement of hair variables is becoming reality whatever the hair-skin diversity and would be valuable as a diagnostic tool that could be employed for drug discovery projects and clinical trials.

O-7A-2
Methodology for the Assessment of Efficacy in Clinical Trials of Cicatricial Alopecia

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Background: There is currently neither effective treatment for the various lymphocyte-predominant cicatricial alopecias nor any established methodology to assess efficacy in clinical trials in this group of disorders. In order to move forward in this area, we need both definitions of the various types of cicatricial alopecias and means of assessing potential treatments.

Methods: The North American Hair Research Society has developed a pathology-driven classification of cicatricial alopecia as well as consensus clinical definitions of each subtype which will facilitate the inclusion criteria for clinical trials in cicatricial alopecia. In an attempt to address the deficiency of methods to assess the clinical severity of these conditions, several new methods were evaluated in a study of three patients with lichen planopilaris (LPP) treated with efalizumab, a humanized immunoglobulin (Ig) G1 version of
the murine efalizumab monoclonal antibody MHM24 which recognizes human CD11a. These methods addressed both the investigator assessment of extent of hair loss and level of scalp inflammation, subject assessment of pruritus and pain and scalp biopsy assessment of degree of inflammation during the course of treatment with a biologic response modifier.

**Results:** The difficulties in assessing cicatricial alopecia in this study as well as in clinical practice and recommendations for a Cicatricial Alopecia Area and Severity Index (CAASI) score that can be used in clinical trials of various types of cicatricial alopecia will be presented.

**O-7A-3**

**The Role of Scalp Dermoscopy in the Diagnosis of Alopecia Areata Incognita**

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**Introduction:** Alopecia areata incognita describes a variety of alopecia areata characterized by acute diffuse hair shedding without typical patches. The condition is frequently misdiagnosed as telogen effluvium.

**Objectives:** We report 70 cases of alopecia areata incognita. The patients (12 M and 58 F, mean age 33.37 years) were diagnosed at the Department of Dermatology, University of Bologna and at the Department of Dermatology, University of Catania during the period 2002-2006.

**Approach:** All the patients were evaluated clinically and with video-dermoscopy. We describe also the pathological features of 50 patients

**Results:** Clinical features showed diffuse hair thinning; in 23 patients hair thinning was more severe on the androgen dependent scalp. Dermoscopic features showed short regrowing hairs together with numerous, diffuse, round or polycyclic yellow dots.

The video-dermoscopy findings were correlated and supported by the histological features of the scalp specimens that showed an increased number of vellus hair follicles and a slight increase in telogen follicles. Follicular stelae were raised. Adequate or slight reduced number of terminal anagen hairs was observed. Telogen germinal units were present. Subtle peribulbar lymphocytic infiltrate was often seen only around vellus anagen hair follicles in the papillary and in the middle dermis. Mild fibrosis was observed around the infundibulum and isthmus level of the follicles.

**Conclusion:** Video-dermoscopy is a first aid before performing the biopsy and can help the clinician to find the right place to perform the sample, but it can also avoid unnecessary biopsies. The device is finally useful to follow the disease during and after treatment.

**O-7A-4**

**Visualizing Hair Follicle-Associated Lymphatics in Human and Murine Skin**

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In our pursuit to understand mechanisms that influence hair growth and the hair cycle we initiated studies on the lymphatic system associated with the hair follicle. The hair follicle undergoes extensive vascular remodeling though out its growth cycle. However, there is a paucity of knowledge regarding the role of lymphatics, specialized endothelial cells, in hair biology or hair diseases.

We quadruple-stained thick (60-180 microns), fixed normal human scalp and murine dorsal skin sections and imaged them using laser scanning confocal microscopy. Human tissues were stained with DAPI (nuclear marker), Ulex-europeaeas-fiTC (vessels and hair follicle keratinocytes), PGP9.5 (pan-neuronal marker) and LYVE-1(lymphatics). Mouse dorsal skin was stained with DAPI, CD31 (vascular marker), PGP9.5 and LYVE1. LYVE1 recognizes vascular endothelial hyaluronan receptor 1 lymphatic receptor for the extracellular matrix mucopolysaccharide hyaluronan.

We found robust LYVE1-immunoreactivity (ir) of well-defined tubular structures that run parallel to the lower portion of the anagen hair follicle. In the mouse skin these lymphatics appear to be open-ended and not in intimate contact with the follicle. In the human skin however the lymphatics run directly adjacent to the lower portion of the hair follicle, below the bulge region and LYVE1-ir is detected in some smaller vessels in the dermis as well.

These imaging studies provide the foundation to examine the role of lymphatics in normal hair cycling in human and model systems and could provide unique insights into our understanding of the immune privilege of the hair follicle and/or loss thereof in the disease state.
Comparison of Hair Growth Parameters in Pre- and Post-Menopausal Women Using a Digital Macrograph Imaging System

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Hair loss is a distressing, common condition for which patients frequently seek medical attention. Post-menopausal women have for many years wondered if reduced estrogen levels could negatively impact their hair.

Objective: To determine if post-menopausal women have different hair density and growth rate compared to pre-menopausal women.

Approach: Thirty eight women between 18 and 65 without overt hair or scalp disease or hormonal disorders were recruited. On each subject approximately 5 cm² Occipital and Frontal sites were chosen, the hair clipped to 1mm, and a temporary tattoo marked. A 0.8 x 1.0 mm area was imaged with an analog high-scope. After 24 hours, a second image was collected. A trained image grader identified and measured each hair in every image.

Results: In all women, the hair growth rate was lower in occipital than frontal scalp. In pre-menopausal women the frontal scalp had higher hair counts than the occipital scalp. In post-menopausal women hair counts significantly decreased in the frontal scalp when compared to pre-menopausal women, resulting in no difference between frontal and occipital scalp. African American women have fewer hair counts compared to Caucasian women in both frontal and occipital scalp.

Conclusions: There were substantial differences in hair growth characteristics between pre- and post-menopausal women. In pre-menopausal women there was a higher hair density on frontal than occipital scalp, and in post-menopausal women the frontal density had decreased to equal occipital. This indicates that in this study frontal scalp was more influenced by hormonal status than occipital scalp.

Haplotype Analysis Identifies a Key Network in the Pathogenesis of Alopecia Areata in Mice

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Alopecia areata (AA) is a cell mediated autoimmune disease targeting anagen stage hair follicles. Two inbred mouse strains, C3H/HeJ and A/J, develop an AA-like disease. Four (Alaa1-4) quantitative trait loci (QTL) intervals were defined in the C3H/HeJ AA model. Haplotype analysis screened single nucleotide polymorphisms (SNPs) within the 4 Alaa1-4 QTL intervals by searching for groups of 3 or more contiguous SNPs identical between C3H/HeJ and A/J but different for C57BL/6J and DBA/2J strains. This approach identified the transporter 2, ATP-binding cassette sub-family B (Tap2) gene as one of the genes within the most significant QTL (Alaa1) on chr. 17. This gene is part of a network involved in CD8 T cell activation. To determine whether there was concordance between haplotype-mapped QTL and gene expression QTL (eQTL), gene expression studies (Affymetrix) were performed on skin from mice receiving skin grafts from affected vs. normal mice at 5, 10, 15, and 20 weeks after grafting as well as for mice with spontaneous AA vs. normal skin. These studies revealed a steady and significant increase of over 3 fold in Tap2 and Tap binding protein (Tapbp) expression, in addition to the altered expression of many other genes in the MHC class I antigen presentation and effector pathways. These studies suggest that allelic variation in MHC genes associated with antigen presentation may confer risk for AA in mice. The homologous genes in humans are located on chr. 6, where a QTL for AA susceptibility was recently mapped.

Retinoic Acid Synthesis and Degradation Enzymes and Binding Proteins Are Altered in Alopecia Areata

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Alopecia Areata (AA) is a non-scarring hair loss that affects 0.1-0.2 percent of humans. To better understand this disease, AA was induced by grafting full thickness skin samples from either AA or normal mice onto young normal C3H/HeJ mice. Skin was collected at 5, 10, 15, and 20 weeks after surgery for Microarray analysis using the
Alopecia Areata in Scotland – Results of a Questionnaire Study

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Epidemiological data on predisposing events, progression and management of alopecia areata (AA) are lacking. We constructed a questionnaire to examine these factors in patients with AA and sent it to all members (n=220) of Alopecia Help and Advice Scotland (AHAS), a patient support group based in Scotland's central belt. 66/220 (30%) affected individuals replied (80% Female, 20% males; age range 9months-88yrs). 79% described the onset of their alopecia as a focal patch of scalp involvement, 15% presented with diffuse alopecia and 2% presented with alopecia universalis. 62% of patients identified a predisposing event. The majority of patients were seen by their GP within a week (67%), 15% were seen by a dermatologist within 4 weeks but 3 waited over a year. 21% reported co-existent autoimmune disease and 30% co-existent atopy, 48% had a family history of autoimmune disease. 17/50 women had a pregnancy while suffering from AA during which 64% experienced an improvement and 24% a deterioration. 62% of the patients wear a wig routinely, 53% use prescription wigs, 15% self finance and 32% do both. The majority have spent over £1,000 on wigs and hairpieces with 16 having spent over £1,000 on alternative therapy. 23% spent £1,000 on alternative therapy. Although the ascertainment rate for this questionnaire was low (24%), the data are revealing. A deeper understanding by doctors of the issues important to these individuals may help in supportively managing this condition.

Acknowledgement: We are grateful to AHAS for allowing use to use their database.

O-7B-4
Genomewide Scan For Linkage Reveals Evidence of Several Susceptibility Loci For Alopecia Areata

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Alopecia areata (AA) is a genetically determined, immune-mediated disorder of the hair follicle with a lifetime risk of approximately 2%, making it one of the most common autoimmune diseases. It is defined by a spectrum of severity that ranges from patchy localized hair loss on the scalp to the complete absence of hair everywhere on the body. In an effort to define the genetic basis of AA, we performed a genomewide search for linkage in 20 families with AA. Our analysis revealed evidence of at least four susceptibility loci on chromosomes 6, 10, 16 and 18, by use of several different statistical approaches. fine-mapping analysis with additional families yielded a maximum multipoint LOD score of 3.93 on chromosome 18, a two-point affected sib pair (ASP) LOD score of 3.11 on chromosome 16, several ASP LOD scores >2.00 on chromosome 6q, and an HRR LOD of 2.00 on chromosome 6p in the region of the MHC locus. Our findings confirm previous studies of association of the MHC locus with human AA, as well as the C3H-HeJ mouse model for AA. The major loci on chromosomes 16 and 18 coincide with loci for psoriasis reported elsewhere and the locus on chromosome 18 corresponds to a region that shows linkage to hereditary hypotrichosis simplex. Our results suggest that these regions may harbor gene(s) involved in a number of different skin and hair disorders.
**O-7C-1**

**Odor Restores Hair Cycle Delay Caused By Immobilization Stress**

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Recent studies have suggested that stress induces hair loss. The aim of this study was to examine the effects of immobilization stress upon hair cycle and to determine whether odorant exposure may restore the effect of stress. Adult female mice of C57BL/6 were administered immobilization stress for 1 – 7 days. Immobilization stress of 3 – 7 days delayed anagen entry and prolonged resting phase (telogen) in mice, consistent with the effect of other stress models. We then examined effects of valerian oil, since valerian root oil has been reported to have sedative effects in both human and mice. The ratio of hair follicles in active phase (anagen II) was significantly increased in the mice applied immobilization stress under the exposure of valerian odor, compared with that in mice exposed to non-odor vehicle triethyl citrate, showing that stress-induced hair cycle was restored by exposure to valerian root oil. We then counted the number of degranulated mast cells. The number was decreased in mice under valerian odor exposure, suggesting that immunoresponse were involved in this stress model and that odorant exposure restored it. All these results suggest that valerian root oil odor could affect immunoresponse and restore the hair cycle delay under the emotional stress.

**O-7C-2**

**Stress Response in a Mouse Model of Alopecia Areata**

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Stress has been suggested to play an important role in the development of alopecia areata (AA). We investigated the effects of stress in the C3H/HeJ AA mouse model. Normal (n=36) and AA affected (n=36) mice were examined before and after exposure to physiological (ether exposure) or psychological (physical restraint) stress conditions. Blood was evaluated by radioimmunosorbent assay while lymph nodes, skin, hypothalamus, pituitary gland and hippocampi were evaluated by quantitative PCR. AA mice had significantly blunted corticosterone (CORT) and adrenocorticotropic hormone (ACTH) responses to acute ether stress, but not to restraint stress. After repeated restraint stress, CORT responses of normal mice decreased due to habituation, but elevated CORT levels persisted in AA mice and hippocampal glucocorticoid receptor (GR) expression increased almost 2-fold. AA mice had higher Proopiomelanocortin (POMC) levels in the pituitary at basal and chronic stress conditions (p<0.05), showing a more activated hypothalamic-pituitary-adrenal (HPA) axis compared to controls. In skin, POMC and corticotrophin receptor 2 significantly increased in AA mice compared to controls (p<0.05), while corticotrophin receptor 1 and GR expression levels were decreased, consistent with active peripheral HPA activity and negative feedback. These results indicate that AA mice have significantly blunted responses to acute physiological stressors and less adaptive responses to repeated psychological stressors. This failure to respond to acute physiological stress suggests a possible increase in vulnerability to pro-inflammatory activity, which may play a role in the pathogenesis of AA. The failure to habituate to repeated psychological stress may also indicate an inadequate capacity for adaptation.

**O-7C-3**

**Altered Peripheral Nerve Function in Alopecia Areata**

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Alopecia areata is an immune-mediated skin disease. For over a century, it has been suggested that alopecia areata is also influenced by the nervous system. Recent studies have shown that some patients prominently express Substance P (SP) in affected scalp skin. Low serum levels of Calcitonin Gene Related Peptide (CGRP) have also been reported, as has an exaggerated vasodilatory response to local injection of CGRP. Moreover in long-standing (>2 years duration) extensive alopecia areata perifollicular innervation appears condensed and arranged differently as compared to normal anagen follicle innervation. These observations have led us to explore whether patients with alopecia areata demonstrate normal peripheral nerve function. Scalp nerve function was tested on 39 control subjects and 20 patients (9M, 11F), ages 24-65, who had either patchy or extensive scalp alopecia areata for an average of 15 years. Sensory nerve conduction threshold (sNCT) for three sensory fiber subtypes: A-beta (2000 Hz), A-delta (250 Hz) and C fibers (5 Hz) was determined using transcutaneous electrical stimulation with the Neurometer CPT (Neurotron, Inc. Baltimore, MD). Dermatomes C2, C6 and Trigeminal V1 were studied. In patients with patchy disease, sNCT measurements were taken from both affected and unaffected areas of the scalp and only
from one site in patients with extensive hair loss. Sensory nerve abnormalities were detected in the C6 dermatome among the smaller diameter fibers in the alopecia areata patients when compared to controls. Both A-delta and C-fiber thresholds were altered (P<0.05), corresponding to a hyperesthetic state compared with healthy controls. These results support the hypothesis that peripheral nerve function is altered in alopecia areata, particularly in patients with long standing disease.

O-08-1
Processing of Proopiomelanocortin in Melanocytes of the Human Hair Follicle and Epidermis – Implications For Regulation of Melanogenesis

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Pro-opiomelanocortin (POMC)-derived peptides adrenocorticotropic hormone (ACTH) and a-melanocyte-stimulating hormone (a-MSH) are widely recognized to be the principle mediators of epidermal pigmentation, via their action at the melanocortin-1 receptor (MC-1R). We have recently reported a similar system, which also includes b-endorphin and corticotrophin-releasing hormone (CRH), regulates hair follicle melanocyte (HFM) biology in vitro. POMC peptide function however, is thought to depend critically on degree of POMC processing to its cleavage products.

Epidermal (EM) and HFM were established from normal human hair scalp (n=5), and POMC and POMC-derived peptides were assessed in conditioned medium (before and after CRH stimulation) using immuno-radiometric assays. POMC-processing enzymes (PC1, PC2, 7B2), MC-1R and CRH-R1 and -R2 were detected immuno-cytochemically. We found that POMC was secreted by both human EM and HFM at relatively similar concentrations. By contrast, ACTH and a-MSH were not released (except for HFM of a single donor), despite expression of the POMC processing machinery in these cells. CRH stimulation of HFM increased both POMC (50-fold) and a-MSH (22-fold) but did not affect ACTH. In parallel studies POMC was found to be an agonist at the MC-1R and was able to stimulate melanocyte dendricity, proliferation and melanin content.

This study is the first to report the secretion of POMC from human HFM and EM in vitro. Despite its incomplete processing to ACTH and a-MSH, POMC exhibited functional activity at the MC-1R, and was further upregulated by CRH stimulation. These findings highlight the importance of POMC processing as a key regulatory event in HFM and EM biology.

O-10-1
Focal Atrichia

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Background: The term focal atrichia has been coined for the pencil eraser size areas of baldness frequently seen in the central scalp of women with female pattern hair loss. To determine the frequency of this finding in women with a variety of types of hair loss and the histopathologic correlation, an IRB approved study was conducted.

Methods: 248 women over 18 years old seen sequentially in the Duke University Hair Disorders Clinic had their particular type of hair loss and whether focal atrichia was present determined by Dr. Olsen. Twenty-one women with focal atrichia each underwent two scalp biopsies, one of an area of focal atrichia and one of an area of hair thinning but without focal atrichia. Biopsies were sent in a blinded fashion as to area of origin to Dr. Whiting who performed the histopathological evaluation. Biopsies were divided horizontally, processed routinely and sections stained with hemotoxylin and eosin. Terminal to vellus hair ratios and anagen and telogen percentages were determined. The degree of inflammation and fibrosis were also recorded.

Results and Conclusions: The study has just been completed and data is currently being fully analyzed. The frequency and specificity of the clinical finding of focal atrichia in female pattern hair loss and the histopathological comparison with hair-bearing scalp will be presented.

O-10-2
Female Pattern Hair Loss Revisited: A Pilot Study Suggests Novel Characteristics

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Background: Several patterns of female pattern hair loss (FPHL) are classically recognized: 1) central thinning; 2) frontal accentuation; and 3) male patterns.

Objectives: 1) To determine if current models for FPHL capture the range of patterns observed. 2) To identify other features that may be clinically meaningful.

Approach: Volunteers with untreated FPHL were interviewed and examined, and appropriate lab work-up performed.
**O-10-3**

**Comparison of Senescent and Androgenetic Alopecia Using Microarray Analysis**

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It has been suggested that senescent alopecia (SA) is a different entity than androgenetic alopecia (AGA) despite similar histopathology, since the age of onset, pattern of hair loss and hormonal involvement differ. Microarray analysis of pooled scalp biopsies from three groups of men aged 60 and older was undertaken. Group 1-Controls-no visible hair thinning. Group 2-SA-diffuse hair thinning after age 60. Group 3-AGA: male pattern hair thinning prior to age 40. Affymetrix Human-U133B was used and data analyzed with GCOS and GeneSpring. In AGA, genes involved in epithelial signal to dermal papilla (FGF5), actin cytoskeleton (DST, ACTN2, TNNI3, and PARVB) and mitochondrial function (JAK2, PRKD3, AK2, TRAP1, TRIO, ATP12A, MLL4, STK22B) were downregulated, while oxidative stress and inflammatory response genes were upregulated. Thus, follicular downsizing in AGA is due to decreased molecular signals of anagen onset and maintenance and increased catagen and telogen inducers. While SA is likely the result of decreased signals between the dermal papilla and stem cells required for anagen onset. These data suggest that AGA and SA are distinct clinical disorders with a final common phenotype of follicular downsizing.

**O-10-4**

**The Role of the Androgen Receptor Gene CAG Repeat Polymorphism and X-Chromosome Inactivation Pattern in Postmenopausal Female Pattern Hair Loss**

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Androgens exert their effect via the androgen receptor (AR) gene, this contains a highly polymorphic trinucleotide repeat (CAGn). The length of this repeat affects both AR expression and function. The number of CAG repeats inversely correlates with androgen levels and has been associated with hirsutism and male balding.

This study aims to investigate the role of the CAG repeat polymorphism of the AR gene and the pattern of X-chromosome inactivation in postmenopausal hair loss. 185 postmenopausal females ≥45 were examined and assigned a grade for hair loss. Subjects also completed a questionnaire of subjective scalp hair loss.

Genomic DNA was analysed for CAG repeat length. X-inactivation status was analysed by assessing methylation status with the restriction enzyme Hpall. Spearman correlation was applied to assess the relationship between CAG length and hair pattern grading.

Ludwig pattern hair loss positively correlated with shorter CAG repeat length in women over the age of 65 (p<0.05). X-inactivation analysis showed skewing toward the shorter allele in this group (p<0.05). There was no significant correlation between frontal hair recession or diffuse generalized hair loss and CAG repeat length. Subjective scalp hair changes did not correlate with CAG repeat length.

To our knowledge, this is the first study to identify an association between shorter CAG repeat or the AR gene and skewing of X-inactivation in postmenopausal females. Results were statistically significant in females over age 65. As androgen levels gradually rise after the menopause, at
this age range levels may be sufficiently raised to provoke hair loss in genetically predisposed individuals.

O-11-1
Histopathologic Evaluation of Cicatricial Alopecia: Lessons From a Blind Study of 109 Clinically-Defined Cases

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Primary cicatricial alopecia (CA) represents a group of entities with overlapping clinical morphology in which biopsy is often utilized as a diagnostic aid. Based on previous work, we believe that CA is best subdivided into lymphocyte-mediated (LM) and neutrophil-mediated (NM) forms. Despite the delineation of many microscopic criteria, including features that are reputedly specific, diagnosis by biopsy remains challenging. In this study, we sought to determine if certain microscopic features are indeed specific for certain diseases. We evaluated 198 biopsies from 109 patients (198/109) with clinically unambiguous disease, including 70/30 of lichen planopilaris (LPP); 41/30 of central centrifugal alopecia (CCA); 22/13 of frontal fibrosing alopecia (FFA); 11/7 of pseudopelade; 46/24 of folliculitis decalvans; 8/5 of tufted folliculitis, and 31 control biopsies of non-scarring alopecia. We scored 25 unique attributes and were unable to identify characteristics with diagnostic specificity. findings such as epithelial squamotization, preferential involvement of miniaturized follicles, and premature desquamation of the inner sheath, reputedly characteristic of LPP, FFA and CCA, respectively, were seen in other types of CA. Interestingly, a heavy neutrophilic infiltrate was uncommon in NM CA, but surrogate features such as a plasmacyte-rich infiltrate, extra-adventitial inflammation and fibrosis, and extensive compound follicle formation were much more common in NM CA than LM CA. We contend specific diagnosis of CA requires precise clinical correlation. However, CA can be readily stratified into LM and NM forms in the absence of insightful clinical guidance. We believe this approach produces information that facilitates clinical management.

O-11-2
Successful Hair Regrowth With Early Treatment of DLE Cicatricial Alopecia

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Objectives: We report a case of successful treatment of discoid lupus erythematosus (DLE) of the scalp. A 37-year-old male patient was referred to our clinic for the evaluation of hair loss in 2006. The patient first noticed hair loss on the vertex and right temple of the scalp 8 months before presenting at the clinic. The lesions were initially pruritic. The patient had been treated with topical corticosteroids.

Approach: On examination the patient showed erythematous to violaceous plaques with scales and follicular hyperkeratosis, 1 – 3 cm in diameter. Dermoscopy showed a lack of ostia in some areas. His review of systems was negative for skin disease or other illnesses. Blood and urine analysis were within normal limits. KOH scalp test for fungus was negative. A baseline ophthalmologic examination was also completed.

Two 4-mm punch biopsies were taken for histopathological analysis as well as for immuno-fluorescent staining. Pathology and immuno-histopathology were consistent with DLE.

Treatment: Oral Prednisone 40 mg once daily, tapering by 5 mg/week over 8 weeks; Hydroxychloroquine 200 mg twice daily; topical Clobetasol lotion twice daily; intralesional Triamcinolone injections 10 mg/cc every 4 weeks.

Results: Four months after initiation of the therapy 80% of hair regrowth was observed in all lesions

Conclusion: Hair loss in early DLE of the scalp is reversible. Early diagnosis and aggressive, multi modal therapy is curial to prevent cicatricial alopecia in DLE.

O-11-3
A Case Series of 29 Patients With Lichen Planopilaris – The Cleveland Clinic Foundation Experience on Evaluation, Diagnosis and Treatment

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Background: Lichen planopilaris results in scaling, atrophy, and permanent alopecia with scarring and is thought to be autoimmune in origin.

Objective: To evaluate the clinical findings of patients with lichen planopilaris so as to aid in the evaluation and diagnosis of the disease and to review the current effective therapies.

Approach: We reviewed the medical records of 29 patients with lichen planopilaris that were seen in the Department of Dermatology at The Cleveland Clinic Foundation between 1992 and 2003.

Results: Good responses in the active perimeter were seen with topical steroids, intralesional steroids and tetracycline
and in the inactive end stage with hair transplants and scalp reductions. 

Limitations: This study was limited by being retrospective in nature. 

Conclusion: Although topical high potency and intralesional corticosteroids remain the mainstay for treatment of lichen planopilaris, the use of tetracycline in this disease may be more helpful than once thought. 

O-11-4 
Clinical Spectrum of Postmenopausal Frontal Fibrosing Alopecia 
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Postmenopausal frontal fibrosing alopecia was described by Steven Kossard in 1994. It is thought to be a special manifestation of lichen planopilaris. In the last years, we have classified more than 20 patients to suffer from this scarring alopecia. The spectrum seems to be quite variable with:
• some patients showing not only frontal but also circumferential alopecia, enlarging in a centripetal pattern
• one patient being a man and not a postmenopausal woman
• some women additionally showing a pronounced vertex alopecia
• different speeds of progression. 
This spectrum will be presented and discussed. 

O-11-5 
Retinoic Acid Synthesis Enzymes and Binding Proteins Are Increased in Central Centrifugal Cicatricial Alopecia 
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Little is known about the etiology and pathogenesis of Central Centrifugal Cicatricial Alopecia (CCCA). Retinoic acid (RA), the active form of vitamin A, is used as a topical therapeutic agent for many dermatologic disorders. We found a complete system of enzymes, binding proteins, and receptors involved in RA synthesis and signaling localized to the hair follicle, sebaceous gland, and epidermis in a hair cycle-dependent manner (Everts et al. 2007 in press). Analysis of this system of RA synthesis in skin affected by cicatricial alopecia provides clues as to the etiology and pathogenesis of this disfiguring disease, provides markers to distinguish CCCA from other primary cicatricial alopecias, and predicts novel therapeutic targets. We performed immunohistochemistry with antibodies against cellular retinol binding protein (CRBP), retinol dehydrogenase (DHR59), retinal dehydrogenases 1-3 (ALDH1A1, ALDH1A2, ALDH1A3), and cellular retinoic acid binding protein 2 (CRABP2) on human skin samples of CCCA and asebia mutant mice (Scd1^{+/-}, Scd1^{a/b2}), a model for human CCCA, and their respective controls. Overall, immunoreactivity of RA synthesis enzymes and binding proteins were more intense in CCCA and the mouse model than their respective controls, with a few exceptions. This result was more significant in the outer root sheath of the isthmus with Dhrs9, and the epidermis with ALDH1A1, ALDH1A2, and ALDH1A3. Mouse strain differences in Dhrs9 expression were identified and Scd1^{a/b2} mice displayed more sex dependent differences. Since toxic RA leads to follicular dystrophy similar to early changes in CCCA, local RA toxicity may be key to the pathogenesis of CCCA. 

O-12-1 
Topical Application of Antagonist to the G-protein Coupled Receptor Smoothened of the Sonic Hedgehog Signaling Pathway Inhibits Hair Growth in C3H Mice 
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The sonic hedgehog (Shh) signaling pathway plays pivotal roles in hair morphogenesis during embryogenesis and hair shaft elongation and cycling in the adult. Recently, synthetic small molecule agonists and antagonists to Smoothened, a G-protein coupled 7-transmembrane receptor protein (GPCR) of the Shh signaling pathway, have been identified. Agonists to Smoothened were previously shown to accelerate anagen entry from telogen follicles in rodents (Paladini, et al). In this study, we investigated the effect of Smoothened antagonists on hair growth inhibition in C3H mice. We observed that topical application of a Smoothened antagonist inhibited depilation-induced hair growth in C3H mice dose-dependently and this inhibitory effect was reversible upon withdrawal of the drug. The extent of hair growth inhibition correlated with drug concentrations in the skin and down-regulation of the Shh signaling pathway genes (Gli1, Ptc1, Gli2, etc) and the hair
growth marker gene (K6irs) normally accompanied with the depilation-induced hair cycle. Morphologically, hair growth inhibition was associated with reduced size of the follicles and aberration of differentiation to form hair shaft. Immunohistochemistry staining revealed that the antagonist led to dose-dependent inhibition of cell proliferation (Ki67) and reduction in the expression of follicle differentiation marker genes (K31 and K73, markers for hair cortex and the inner root sheath, respectively). However, hair growth inhibition was accompanied by an absence of detectable caspase-3 staining in the hair bulb indicating these follicles remained in anagen. These results suggest that small molecule antagonists to Shh signaling pathway may provide potential therapeutic modalities for treating dermatological conditions such as unwanted body hair in the skin.

O-12-2
The miRNA Processing Enzyme Dicer is Required for Hair Follicle Maintenance in Adult Skin

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MicroRNAs (miRNAs) are endogenous approximately 22 nt RNAs that regulate gene expression by binding the 3’ untranslated regions of protein-coding mRNAs, resulting in mRNA cleavage or inhibition of translation. Conserved vertebrate miRNAs are predicted to target more than 400 regulatory genes, suggesting broad roles in biology. We, and others previously demonstrated that multiple miRNAs are expressed in developing and postnatal skin and hair follicles, and that constitutive deletion of the miRNA processing enzyme Dicer in embryonic mouse skin causes failure of hair follicle morphogenesis and subsequent follicular degradation. Here we investigate whether Dicer and miRNA function is also required in established hair follicles in the adult mouse.

For these experiments we generated K5-rtTA tetO-Cre Dicerfl/fl mice in which the Dicer gene can be inducibly deleted in the basal epidermis and hair follicle outer root sheath, including hair follicle stem cells, by dosage with oral doxycycline. Inducible Dicer deletion starting at postnatal day (P) 20, when hair follicles are just entering the first postnatal growth cycle, resulted in loss of external hair starting within 10 days of doxycycline treatment. Histological analysis at P45 revealed that mutant hair follicles remained in an abnormal growth phase, while hair follicles in control littermate skin had entered telogen. Expression of the hair follicle stem cell markers K15 and S100A6 was lost from affected mutant hair follicles, despite maintenance of expression of the outer root sheath marker Sox9. Analysis at P70 and P90 showed that the mutant hair follicles degenerated, similar to the phenotype seen in constitutive epidermal Dicer mutant mice.

These data demonstrate that in addition to its essential role in embryonic hair follicle morphogenesis, Dicer function is required in mature skin for normal timing of the hair follicle growth cycle, and for maintaining the hair follicle epithelial stem cell population and follicle integrity.

O-12-3
The Autoimmune Regulator (AIRE) 7215C Allele Is Strongly Associated With Failure of Diphencyprone (DPCP) Treatment in Alopecia Areata (AA): Prospect of Developing a Genetic Test to Predict Therapeutic Response

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Objectives & Approach: Success rates for diphencyprone (DPCP) therapy of alopecia areata (AA) vary widely between published series. There is general agreement that hair regrowth is less likely in patients with longstanding or severe disease (alopecia totalis/ universalis) but it is very difficult to assess prospectively which patients will actually respond to this treatment.

Mutations of the autoimmune regulator gene (AIRE) cause the rare autoimmune polyendocrinopathy type I syndrome (APS1) in which alopecia areata occurs with a frequency of up to 60% of cases. We have previously demonstrated significant association with AIRE variants in a large series of 290 AA cases not associated with APS1. We have now analysed a subgroup of these patients, 61 of whom have received diphencyprone therapy for their AA, comparing AIRE variant genotypes between cases with successful regrowth stimulated by DPCP and those with no regrowth. Published series. There is general agreement that hair regrowth is less likely in patients with longstanding or severe disease (alopecia totalis/ universalis) but it is very difficult to assess prospectively which patients will actually respond to this treatment.

Conclusions: With further refinement, these results open the possibility of using a prior genetic test to assess the likelihood of success with DPCP which although well worthwhile in successful cases, is an inconvenient, expensive and unlicensed treatment.
O-13A-1

Pili Annulati-Reduction of Candidate Region to 2.9 Mb By Genetic Analysis of 4 Additional Families With Pili Annulati and Expression Analysis of Genes in the Critical Region

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Background: Pili annulati is an autosomal dominant transmitted hereditary hair disorder characterized by alternating light and dark bands in the hair fibre of affected individuals. Recently, a locus for pili annulati was mapped to chromosome 12q24.32-24.33 by linkage analysis in 5 families segregating this trait. Recombination events defined a critical region of 8 Mb.

Objectives: The aim of the current study was to reduce the size of the candidate region by analysis of further families and to investigate the expression of possible candidate genes in hair follicles and scalp tissue.

Approach: Genomic DNA was extracted from 96 individuals of 4 families, after examination to establish their phenotype. Finemapping was performed in all 96 individuals using 26 microsatellite markers spanning a 20 cM region at the telomeric end of chromosome 12. Candidate genes were analyzed for their expression in hair follicles, derived from plucked hair follicles, scalp and other tissues by RT-PCR.

Results: In family I, 7 individuals were affected, 5 unaffected. The largest family so far described for pili annulati in the literature was family II, with 26 affected and 39 unaffected family members over 3 generations. Family III and IV were smaller families with 3 and 6 affected and 8 and 2 unaffected individuals, respectively. In family I and family II recombinations were identified which reduced the region by more than half from 8 Mb to 2.9 Mb containing 38 known and putative gene loci. We have analysed a majority of the genes in this region by RT-PCR and have found that 21 were expressed in plucked hair follicles.

Conclusion: In summary we confirmed the locus for pili annulati in 4 further families, reduced the critical interval to 2.9 Mb, and identified possible candidate genes expressed in the human hair follicle.

O-13A-2

Dominant Mutation in the Rod Domain of Keratin 6hf Results in Hair Phenotypes Resembling Trichorrhexis Nodosa in Mice

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The keratin 6hf genes, KRT6HF for human and Krt6hf for mouse, are expressed along the entire companion cell layer and the upper matrix region of anagen hair follicles. It is also the only type II soft keratin that is expressed in the medulla of the hair shaft. The introduction of a point mutation at the beginning of the helical rod domain of the mouse Krt6hf gene (N158Del), which corresponds to the “hot spot” mutation in KRT6A (N171Del) that causes pachyonychia congenita, resulted in congenital and inheritable hair shaft breakage in mutant mice. The transverse breakage of pelage hair was caused by bulbous swellings along the hair shaft. Therefore, this mouse model grossly mimics trichorrhexis nodosa, one of the most common hereditary hair fragility syndromes in humans. More interestingly, this bulbous hair phenotype could be recapitulated when the epidermal keratinocytes from newborn mutant mice were grafted onto immune compromised nude (Foxn1nu) mice. Therefore, this mouse model is an ideal in vivo tool to study the disease mechanisms underlying trichorrhexis nodosa, and to test novel therapeutic strategies to treat this disease. A lentivirus delivery system is currently being developed to express a mutation-specific siRNA. We expect that this siRNA, when delivered to keratinocytes of the mutant mice, will be able to suppress the recurrence of the bulbous hair phenotype.

O-13A-3

Matrix to Intermediate Filament Ratio in the Cortex of Merino Wool Correlates to Curl

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Intracellular intermediate filaments (IFs) are the fundamental building blocks of the hair, wool or fur of mammals. In the cortex of mammalian fibres IFs, surrounded by a globular matrix, form bundles called macrofibrils. The cortex of wool is made up of three cell types (orthocortex, paracortex and mesocortex) that are defined by the arrangement of IFs within macrofibrils. The distribution of cells of different types in a wool fibre is associated with direction of curvature, with orthocortex cells being typically found on the edge of the fibre corresponding to the outside of the curl, paracortex on the inside and mesocortex centrally. In addition to IF arrangement, macrofibrils of the
different cell types appear to vary in the amount of matrix they contain. Having an accurate measure of the proportion of matrix in the macrofibrils of different cell types is valuable for building biologically realistic single-fibre mathematical models that can be used for predicting fibre behaviour following structural/chemical damage or modification. We used Fast Fourier transform image analysis to obtain data on IF spacing from selected regions of high-magnification electron tomograms and then, using geometrical methods, we calculated matrix to IF ratios. The proportion of matrix was significantly different in the three cell types. Paracortex cells had the most matrix (0.61), orthocortex had the least (0.42) and mesocortex were intermediate (0.54). This leads to a pattern of more matrix material on the inside of the curl and less on the outside and vice versa for IF material.

O-13A-5
Hair Photoaging: Ultraviolet Induced Photodegradation and Restoration of Human Hair
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Objectives: Human hair is constantly subjected to repeated environmental assaults, commonly termed weathering. Among the various sources of hair damages, it is well known that exposure to ultraviolet(UV) radiation damages hair fiber. UV light induced hair photoaging is difficult to avoid during daily life. We performed this study to observe the photodegradation and restoration pattern of human hair.

Approach: We studied the morphological changes of hair after UV irradiation sequentially with scanning and transmission electron microscope. We also checked the soluble hair protein released from damaged hair with protein analysis, electrophoresis and western blot analysis. Then, we studied the changes of integral hair lipid present in the cell membrane complex(CMC) in hair cuticle with lipid transmission electron microscope and HPLC.

Results: Photoaged hair showed sequential cortical and cuticular alterations and restoration especially severe in the endocuticular layer of hair cuticle. Release of soluble hair protein and partial degeneration of integral hair lipid were also noted and restored gradually by UV irradiation.

Conclusion: UV induced photoaging show morphological and chemical changes of human hair. It also affect alterations of CMC lipids.

O-13A-6
Characterization of Female Facial Hair: Morphology and Growth Properties of Two Novel Subtypes of Upper Lip Terminal Hairs and Responses to Vaniqa Treatment.
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Objectives: To better understand the morphology and growth properties of female facial hair.

Approach: Two clinical studies were fielded to capture growth properties, physical appearance, and one for treatment effects from Vaniqa (13.9% DFMO) compared to vehicle control. 150 pre-menopausal women were recruited for having unwanted facial hair and routinely performing some form of hair management. Growth rate measures were calculated from Hi-Scope image and expert grading of global images from digital photographs. Self assessments were recorded to support technical findings.

Results: Images of 7 days of regrowth consistently showed that femal upper lip hairs could be classified into two types based on diameter and growth rates. Type I hairs had an overall larger diameter and a 2.5X higher growth rate than Type II. While 20% more of total Type I were in anagen compared to Type II, there were about 1.5X more Type II in total hair count. Vaniqa significantly reduced baseline growth rates of Type I by 45% whereas Type II were reduced by 29%. Expert judging of global images and self assessments confirmed the overall effects.

Conclusion: Two morphologically distinct types of female upper lip hairs were found different growth properties and sensitivity to DFMO. This potential difference in response could be that anti-proliferatives will be less effective against slower proliferating cells than higher proliferating ones. It is unclear at present what, if any, relationship there is between Type I and II (Do Type II hairs transition to Type I, etc).

O-13B-1
A Novel Perspective on the Significance of Ferritin in Postmenopausal Hair Loss
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The aetiological role of iron deficiency in female hair loss is an area of controversy. Iron deficiency has been associated with both chronic telogen effluvium and female pattern hair loss. Postmenopausal women form a unique group to study the relationship between iron stores and hair loss due to the lack of menstrual iron loss. This study aims to identify the relationship between serum ferritin and hair loss in postmenopausal women.
198 postmenopausal women underwent a detailed scalp examination and were allocated a grade (1-6) for both Diffuse generalised hair loss (DGL) and Female pattern hair loss (FPHL). Subjects with thyroid disease were excluded. Serum was collected under standard conditions for analysis of serum ferritin. As ferritin levels rise in systemic illness, parallel measurement of C-reactive protein was concurrently undertaken. Spearman rank correlation was applied to analyse the relationship between hair loss pattern and ferritin.

134 women had no hair loss, 38 had FPHL and 26 had DGL. Mean ferritin values were lowest in women with DGL (47±23mg/l) compared to 65±54mg/l for those with no hair loss. Interestingly, females with FPHL had the highest mean value for serum ferritin (97±104mg/l).

There was positive correlation between degree of FPHL and ferritin (p<0.05). Women with DGL had lower levels of ferritin but this was not statistically significant.

This study has found raised serum ferritin levels in postmenopausal women with FPHL. Iron stores increase after the menopause. Recent studies have shown raised ferritin levels to act as a marker of cardiovascular disease risk. The results of this study suggest an alternative aetiology to iron deficiency in FPHL. Further work is required to identify the significance of postmenopausal hair loss and raised ferritin as a marker of systemic disease.

O-13B-2
Iron Deficiency in Female Pattern Hair Loss, Telogen Effluvium and Controls
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Background: The evidence to date remains unclear whether iron deficiency plays a significant role in female pattern hair loss (FPHL) or in telogen effluvium (TE).

Hypothesis: Iron deficiency is more common in women with FPHL and/or telogen effluvium than in age matched controls without hair loss.

Methods: Two part study. Part I: retrospective chart review of female patients with FPHL or TE see in Duke University Hair Disorders clinic who also had laboratory documentation of a serum ferritin and/or hemoglobin. Part II: prospective control study of women ages 18 to 65 recruited from employees and students at DUMC who did not have a history of or physical exam findings consistent with hair loss. Serum ferritin, ESR and hemoglobin were collected in these controls. Iron deficiency was defined by a serum ferritin of < 41ng/dL. Iron deficiency anemia was defined as hemoglobin < 12g/dL. Statistical analysis was performed to determine prevalence of iron deficiency in these populations.

Results: 55% (56/101) of women with telogen effluvium and 48% (72/151) of women with FPHL had iron deficiency as defined above. 9% (5/56) and 4% (3/72), respectively, had iron deficiency anemia. Iron deficiency was seen in 67% (35/52) and iron deficiency anemia in 3% (1/35) of the control population.

Conclusions: Iron deficiency is a common problem in women but is not more common in those with TE or FPHL than those without hair loss. Further investigation is needed to determine if correction of iron deficiency would augment hair regrowth in TE and FPHL.

O-13B-3
Effect of Oral Intake of Choline-Stabilized Orthosilicic Acid on Hair Tensile Strength and Morphology in Women With Fine Hair
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Silicon (Si) has been suggested to have a role in the formation of connective tissue. Choline-stabilized orthosilicic acid ("ch-OSA") is a bioavailable form of silicon which was found to improve skin microrelief and skin mechanical properties in women with photoaged skin.

The effect of ch-OSA on hair was investigated in a randomized, double blind, placebo-controlled study. During 9 months, 48 women with thin hair were given orally either ch-OSA (10 mg Si/day; n=24) or a placebo (cellulose; n=24). Urinary Si excretion, hair morphology (apparent diameter, cross sectional area), and tensile strength (elastic gradient, break load, break stress) were analyzed.

After 9 months supplementation, urinary silicon concentration increased in the ch-OSA group (p<0.05) but not in the placebo group. The elastic gradient decreased in both groups but the change was smaller in the ch-OSA group (-4.52 %, p=0.027) compared to placebo (-11.86 %). Break load changed in the placebo group (-10.79 %, p<0.0001) but not in the ch-OSA supplemented group. Break stress decreased in both groups but the change tended to be smaller in the ch-OSA group. Both the apparent diameter and the cross sectional area increased in ch-OSA supplemented subjects (p<0.05, vs. baseline) but not in the placebo group. The change in urinary silicon excretion correlated positively with the change in apparent diameter and cross sectional area (p=0.023).
This study suggests that hair morphology is influenced by Si intake. Oral intake of ch-OSA had a positive effect on tensile strength including elasticity and break load and resulted in thicker hair.

O-13C-1
Erythropoietin: a New Player in Hair Follicle Biology

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Erythropoietin which is mainly synthesized in the kidney upon stimulation by hypoxia, primarily serves as an essential viability and growth factor for erythrocyte precursor cells. However, there is increasing evidence that EPO/EPO receptor (EPO-R) signaling operates as a potential tissue-protective system outside the bone marrow. Arguing that growing (anagen) hair follicles (HFs) are among the most rapidly proliferating and most damage-sensitive tissues in the human body, we have here explored whether human HFs are sources of EPO expression and targets of EPO-R-mediated signaling. Full-thickness human scalp skin and microdissected human scalp HFs were assessed for EPO and EPO-R expression, and the effects of EPO on organ-cultured human anagen hair bulbs were assessed in the presence or absence of a classical apoptosis-inducing chemotherapeutic agent. Here, we show that normal human scalp HFs express EPO on the mRNA and protein level in situ, up-regulate EPO transcription under conditions of hypoxia, and express transcripts for EPO-R and the EPO-stimulatory transcriptional co-factor, hypoxia-inducible factor-1a (HIF-1a). Although EPO does not significantly alter human hair growth in vitro, it significantly down-regulates chemotherapy-induced intrafollicular apoptosis and changes the gene expression program of the HFs (e.g. upregulation of kinesin light chain kinase and down-regulation of calmeigin transcription [microarray analysis]). The current study points to intriguing novel functions and targets of EPO beyond the erythropoietic system: Human HFs are an extrarenal site of EPO production and an extrahematopoietic site of EPO-R expression. They may recruit EPO/EPO-R signaling e.g. for modulating HF apoptosis under conditions of hypoxia and chemotherapy-induced stress.

O-13C-2
Efficacy of Laser Therapy in Hirsute Iranian Women

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Background: There are several different lasers available for the reduction of unwanted hair. According to the theory of selective photothermolysis, laser and intense pulsed light with wavelengths in the red or infrared region (600 to 1200 nm) are most often used for hair removal. Laser systems and IPL currently approved by FDA for the reduction of hair include: the long-pulsed ruby (694 nm), alexandrite (755 nm), diode (800 nm), and Nd:YAG (1064 nm) lasers and IPL (500 to 1200 nm) sources. These are the main types of hair lasers.

Interval of sessions would be suitable for: Face 4 – 8 wks, Leg 4 – 6 months and, Axilla 6 Wks sequentially.

Methods: The side effects of laser treatment especially in Iranian hirsute women will be discussed: including, burns, pain and vesiculation, which were rare after treatment with either diode or IPL, but we observed them more frequently with the long-pulsed diode system at the higher fluence of 40 J/cm^2 and Alexandrite one. In laser therapy: Anagen follicles only are sensitive, and the more fluences of light energy the more destruction of hair follicles will occur.

Alexandrite and Diode:
As in comparison with Ruby, the amount of absorption in melanin is lower, will be better tolerated in Asians. Thus epidermal damage or hypo pigmentation is lower.

Conclusion: For our patients with lighter skin tone, the results of Alexandrite and Diode lasers have been reported better than darker individuals.

O-13C-3
Novel Function of TGFb1 as a Key Pathogenic Molecule in Androgenetic Alopecia: Potentiation of Androgen Receptor Through Smad3

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We have reported the pathogenic role of TGF-b1 production and activation by androgen in balding dermal papilla cells (bald DPCs) using the coculture system of bald DPCs and keratinocytes. Here, we examined the effect of TGF-b1...
on androgen receptor (AR) transactivation by transient transfection assays of MMTV-luciferase reporter vector. TGF-b1 at 0.2 and 2.0ng/ml increased AR transactivation to 2.5- and 2.7-fold, respectively. Because expression of ARA55, one of AR coactivators, is increased by TGF-b1, we tested the possibility that TGF-b1 potentiates AR through ARA55 by using dominant negative C-terminal fragment ARA55. Rather, the dominant negative ARA55 augmented TGF-b1 (2.0ng/ml) effect on AR up to 5.1-fold. When exogenous ARA55 was overexpressed in bald DPCs, TGF-b1 increased AR activity only to 1.5-fold. Therefore, we suggest that ARA55 constitutively coactivates AR but paradoxically interferes TGF-b1 augmentation for AR. Next, we examined whether Smad3 can mediate the signal from TGF-b1 to AR by reporter assays. The silencing for Smad3 by siRNA in bald DPCs reversed completely TGF-b1 potentiation of AR, demonstrating that this signal is dependent on Smad3. Together, in vivo situation of androgenetic alopecia, TGF-b1 produced by androgen from bald DPCs suppresses epithelial cell growth and causes early catagen induction in a paracrine manner and furthermore enhance androgen sensitivity in bald DPCs in an autocrine manner, recapitulating the reciprocal pathomechanism between androgen and TGF-b1.

O-13C-4
Hair Follicles Express Functional Hypothalamic-Pituitary-Thyroid (HPT) Axis-Related Elements

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Hair follicles (HF) are among the most thyroid hormone-sensitive tissues of mammals. Thyroid hormone synthesis and release are under control of pituitary thyroid-stimulating hormone (TSH) and pituitary TSH release, in turn, is under control of the hypothalamic hormone, thyroid-releasing hormone (TRH), which stimulates pituitary TRH receptors (TRH-R). Recent research has indicated that the expression of TRH and TSH is not confined to hypothalamus and pituitary gland, and transcripts for several elements of the hypothalamic-pituitary-thyroid (HPT) axis have recently been identified in cultured human skin cells. Therefore, we intended to clarify whether normal human scalp HF express elements of the HPT axis in situ. By quantitative PCR, specific products were identified for TSH, TSH-R, TRH and TRH-R. Cryostat sections of normal human scalp skin were stained for TRH, TSH and TSH-R. Immunoactivity of the member of the HPT axis was localized to several compartments of human skin and HF. Stimulation of TRH-R in organ-cultured human scalp HFs by TRH resulted in increased TSH production and transcriptional modulation of several genes (e.g. neurofilament 3, keratins [microarray]). TSH treatment evoked significant cAMP-release from cultured HF. In addition, transcripts for several thyroid-associated elements (natrium-iodide symporter, thyroglobulin, thyroid transcription factors) were identified in human scalp skin HFs, some of which were regulated by TSH (e.g., up-regulation of thyroglobulin transcription). These data suggest that normal human scalp HFs are extrapituitary sources and extrathyroidal targets of TSH, and express key elements of the HPT, which appear to be linked in a functional, peripheral equivalent of the HPT.
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A Comparative Analysis of Quality of Life for Adolescents Versus Adults Affected by Alopecia Areata

Austin, Stephanie L.; McCarthy-Veatch, Patricia; Kao, Juihsien; Ahrens, Mary; Hordinsky, Maria; Cristiano, Angela; Price, Vera; Norris, David; Duciv, Madeleine; LeRoy, Bonnie

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Adolescents’ perceptions of disease often differ from those of children and adults. Research is needed to investigate adolescents’ unique perceptions and experiences of disease so that health care and genetic counseling services can be better tailored to meet their needs. In the present study, data were obtained for 43 adolescents with Alopecia Areata (modal age = 15 yrs.) who are part of the National Alopecia Areata (AA) Registry. Data included: demographic and disease data from the National AA Registry and responses to the National AA Registry Quality of Life Questionnaire, a measure of five dimensions of functioning. Their data were compared to those of a sample of 45 adults with AA (modal age = 45 yrs.) selected randomly from the Registry. With the exception of age, there were no significant between-group differences for demographics or AA history. Adolescents scored significantly higher than adults (p < .01) on general functioning, spiritual functioning, physical functioning, and emotional functioning, and significantly lower on social and family functioning. Multiple regression analyses indicated that developmental status (adolescent vs adult) was the only significant predictor of scores for 3 of the 5 quality of life dimensions (general, physical, and social/ family functioning). The results suggest that although AA potentially impacts the functioning of all individuals with the disease, adolescents’ perceived quality of life is not a subset of adult functioning. Adolescents have a functioning style specific to their disease and developmental stage. Services need to be tailored specifically for adolescents struggling with AA.

P-102
Cytokine mRNA Expression in the Skin and Lymphoid Organs of the C3h/Hej Mouse Model for Alopecia Areata

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Alopecia areata (AA) is a chronic hair loss disease involving peri- and intra-follicular infiltration by mononuclear cells. Several studies suggest immunomodulatory cytokines expressed by the inflammatory infiltrate not only act as mediators of immunity and inflammation but also regulate cell proliferation and differentiation and, as such, may play an important part in regulating hair growth and AA development. We examined in vivo levels of mRNA of 13 cytokines and chemokines in the skin, draining lymph nodes, spleen, and thymus of C3H/Hej mice with AA and normal haired littermates using quantitative RT-PCR techniques. The levels of IFN-g and IL10 were statistically significantly higher in the skin and lymph nodes of AA-affected mice than in healthy controls while that of IL18 was lower. No statistically significant changes were found for the mRNA expression levels of IL17, IL21 and GM-CSF. Three main subfamilies of chemokines studied were CC chemokines, including RANTES, macrophage inflammatory proteins a and b (MIP-1 a and b), CXC chemokines, including CXCL1 and CXCL10, and CX3C chemokines represented by CX3CL1; all of which were found highly expressed in skin draining lymph nodes and/or the skin of mice with AA. Chemokines are involved in chemotraction and activation of leukocytes to the site of inflammation and in the induction of cytokine production and are thus key determinants of inflammatory reactions and immunity. The fact that all the studied chemokines were expressed at significantly higher levels in AA-affected mice than in controls suggests they may play an important role in AA pathogenesis.

P-103
finasteride for Treatment of Iatrogenic Androgenetic Alopecia In Woman

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finasteride, a type II 5-alpha-reductase inhibitor, has long been proposed to treat female pattern hair loss. Although female pattern hair loss associated with hyperandrogenism can respond to finasteride, there has been no report on its efficacy for rare cases of androgenetic alopecia that develops in women undergoing androgen therapy. In this work, we describe the efficacy of medium-high doses of finasteride (2.5 mg daily) in the treatment of Hamilton type hair loss associated with exogenous androgen supplementation. Despite the continuation of androgen supplementation, by 10 months of treatment, noticeable improvement of hair coverage on the scalp was observed. The symptoms associated hyperandrogenism including hirsutism and a lower pitch of the voice were also improved under the finasteride treatment. In summary, Hamilton type hair loss can develop on female patients under androgen...
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treatment and the hair loss can be effectively treated with medium-high doses of finasteride (2.5 mg daily) despite the continued androgen supplementation.

P-104
Prostaglandin Metabolism in Hair Follicle: PGE$_2$ and PGF$_{alpha}$ Synthesis and Interconversion
Colombe, Laurent; Vindrios, A.; Michelet, J. F.; Bernard, B A.; L’Oreal Recherche, Clichy, France

Prostaglandins regulate a wide number of physiological functions. Recently PGF$_{alpha}$ an analogue such as latanoprost was shown to have a real impact on hair regrowth. The aim of this study was to investigate and describe the expression profile in human hair follicle of prostaglandin (PG) metabolism key enzymes, i.e. Carbonyl reductase-1 (CBR1), microsomal Prostaglandin E synthase-1 (mPGES-1) and microsomal Prostaglandin E synthase-2 (mPGES-2), cytosolic Prostaglandin E synthase (cPGES), the aldoketoreductase AKR1C1 and the Prostaglandin F synthase AKR1C3.

Quantitative RT-PCR evidenced i) the expression of all these enzymes in plucked hair follicles and ii) a higher expression of mPGES-2 and AKR1C3 in female hair follicles. Using Western blot, PGE2 and PGF2 ELISA assays and immunohistochemistry, we observed that most of hair follicle cell types were able to produce PGE$_2$ and/or PGF$_{alpha}$ and synthesized all PG metabolism enzymes. Moreover, a specific distribution in hair follicle was noted for each of these enzymes. These results demonstrated that an active but intricate prostaglandin metabolism could take place in human hair follicle, mainly oriented towards PGE$_2$ synthesis and concentrated in the bulb, including dermal papilla, matrix and keratogenous area. Local production of PGF$_{alpha}$ might equally rely on PGE$_2$ conversion and direct synthesis through AKR1C3/PGFS.

P-105
Comparative Evaluation of Patchy Alopecia Areata Response to Oral Therapy With Either Zinc Sulfate or Vit.B6
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Background and Aims: Alopecia areata is a relatively common problem as it may affect nearly 1% of world population until the age of fifty. The peak incidence will be in adolescence and young adults. Because of large socio-psychiatric impacts on the patients and its unknown etiology, it deserves a significant consideration from therapeutic point of view. Until now, no effective and safe treatment can eradicate the illness, so it is worthy to study the subject.

Methods: 34 patients with patchy alopecia areata, referred to educational skin centers in Isfahan university were recruited and randomized into two treatment groups, provided that they have the inclusion criteria (not to be pregnant, or under any other treatment, not becoming progressive toward totalis or universalis types, over 7 year old) of the study. In case group we tried oral zinc sulfate (1 mg/kg/day), up to 3 months and in controls oral Vit. B6 tablets(2 mg/kg/day) in the same duration. They were followed exactly for responses in the terms of regrowing hair, subjective satisfaction from the treatment and side effects.

Results: After analyzing the data with the aid of chi-square test, in the case group, we got 11.7% complete response, 76.6% partial and 11.7% no response or failure. In the second group (Vit. B6) we reached consecutively to 5.8% complete, 58.9% partial and 35.3% no response.

Conclusion: With regard to the results of statistical analysis in our trial, the response rates of the two study groups have no significant differences from statistical point of view, though the efficacy of treatment with oral zinc sulfate appears to be higher in alopecia areata subjects, with regards to the extracted data.

Key words: Alopecia areata, Zinc sulfate, Oral treatment, Vit B6(pyridoxine)

P-106
Alopecia Areata: a Comparison Among Diverse Therapies
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1. Biomedical Center – Battipaglia (SA), Olevano sul Tusciano, Italy; 2. Second University of Medicine of Naples, Napoli, Italy; 3. Biomedical Center – Battipaglia (SA), Napoli, Italy

Alopecia Areata is a common autoimmune hair disease, caused by an abnormal cell-mediated response against one or more antigens of the distal part of the hair follicle (lower segment).

A polymorphic cellular infiltration in the deep dermis, composed predominantly of T lymphocytes and Langherans’ cells in the peribulbar region is usually detected. The disease can be determined by a lot of diverse factors which cause hair loss in the anagen phase.

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systemic Corticosteroids, Phototherapy (U.V.B 311 nm), Minoxidil, Psychotherapy, PUVA, SADBE.

During a four years study, 146 patients affected with Alopecia Areata involving at least the 75% of scalp were treated. Efficacy of diverse therapies was evaluated both individually and in association. According to other authors we suppose that Alopecia Areata needs a multidisciplinary approach and that a therapy suiting for all the patients doesn't exist. In fact a lot of factors can variously influence the course of the disease.

P-107

Methotrexate in Patients with Chronic Severe Alopecia Areata of the Scalp

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Common features between psoriasis and alopecia areata, including immunologic and therapeutic aspects, suggest that methotrexate (MTX) may have therapeutic value in alopecia areata (AA).

We report an open, single-center, investigator-initiated pilot study to examine the efficacy of methotrexate in subjects with chronic, severe scalp AA. Study enrollees received weekly oral administration of 15 mg of MTX for 24 weeks. Outcome parameters included the Severity of Alopecia Tool (SALT) score as well as investigator and patient assessment of regrowth.

Four eligible patients with 91-95% scalp hair loss due to AA, of a duration from 2-10 years were included. After 24 weeks, one of them had minimal regrowth, with a 2.1% lower SALT score. The second patient had no change of her condition, except for some regrowth of body hair. The third and fourth patient dropped out of the study after 4 months. In the third patient, the SALT score had decreased from 91% to 76.5%, and he noticed minimally improved scalp coverage. In the fourth patient, there was no regrowth. The treatment was generally well tolerated.

We could not show sufficient efficacy of oral, medium dose MTX in severe, longstanding AA in this small pilot study. However, another recently published study suggests some therapeutic value of MTX, especially in combination with oral corticosteroids. A large, controlled study with longer treatment intervals, different administration routes or higher doses may be needed to finally define the role of MTX in the treatment of AA.

P-108

Study of Apoptosis Regulation Process in Alopecia Areata

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To determine the level of apoptosis and proliferative activity of hair follicles (HF) cells in patients with alopecia areata (AA).

Ten scalp skin biopsy samples have been examined: 8 samples with AA and 2 samples from healthy controls.

With a method of immunohistochemical testing using monoclonal antibodies against DDF, bcl-2, p53, Fas, fas-l and Ki67, we have studied cells at the stage of complete apoptosis and the levels of expression of key apoptosis regulator molecules; also, proliferative activity of HF cells has been studied.

Results: A method of immunohistochemical testing with the use of antibodies against single-stranded DNA has revealed the presence of cells at the stage of complete apoptosis among HF keratinocytes, lymphocytes and endothelial cells in biopsy specimens obtained from donors with AA. Apoptotic cells were not found in controls.

At the skin biopsy with AA in matrix cells and in cells of HF inner root sheath (IRS) was identified protein p53; bcl-2-positive cells were revealed in the matrix of HF bulbs. p53 and bcl-2-positive cells were not found in controls.

In the studied skin samples of patients with AA, large amounts of fas-l were revealed in the basal layer of keratinocytes of outer epidermal sheath of the root (OESR) and in the cytoplasm of lymphocytes. In controls fas-l was present in the region of OESR in small amounts and was completely absent in lymphocytes.

Initiator molecule Fas was absent in biopsy specimens of both AA patients and controls.

Examination of proliferative activity of HF cells at the skin biopsy with AA revealed the presence of individual Ki67-positive cells in the IRS.

Conclusion. Disorder of apoptosis regulation has been revealed in patients with AA.

Changes in interactions between apoptosis promoters and inhibitors lead to development of pathologic telogen and a decrease in proliferative activity of HF cells.
**P-109**

**Aging Processes In Human Hair Follicles**

Giesen, Melanie;1 Gruedl, Sabine;1 Holtkoetter, Olaf;1 Schulze zur Wiesche, Erik;2 Petersohn, Dirk;1
1. Phenion GmbH & Co. KG, Düsseldorf, Germany; 2. Hans Schwarzkopf & Henkel GmbH & Co. KG, Hamburg, Germany

To guarantee the growth of strong and healthy hair various specialized cell types in the follicle interact accordantly to a complex set of molecular signals. Biological alterations due to intrinsic or extrinsic stimuli can destabilise this well balanced system, thus effecting hair growth or metabolism. Also aging could be characterised as a disturbance in this perfectly organised machinery.

Albeit the predominant symptom of hair aging, greying, is addressed in a plurality of research activities, age related changes beyond loss of pigmentation remain obscure. It has been reported that hair follicle density, growth rate and hair diameter decline in age, but the molecular events underlying this macroscopic alterations are still poorly understood. Therefore we characterised hair follicles of two volunteer panels (below 25 years, above 50 years) on the molecular level. In this study we show that concordantly to other biological systems the hair follicle undergoes an aging process associated amongst others with a decline in structural proteins such as several keratins or a shift of apoptotic parameters. Those modifications might be aetiological for the reported alterations and providing bioactives fighting these age related changes is a challenge for cosmetic science.

**P-110**

**Evaluation of Free Oxygen Radical and Antioxidant Capacity in Alopecia Areata**

Huh, Chang-Hun;1 Cho, Hyun-Joo;1 Kim, Dong-Seok;2 Kim, Beom-Joon;2 Youn, Sang-Woong;1 Park, Kyoung-Chan;1
1. Seoul National University, Seoul, Korea; 2. Chung-Ang University, Seoul, Korea

Objectives: The pathophysiology of alopecia areata (AA) has not been clearly defined. We tried to analyze the differences of free radical status and antioxidant capacity in the blood between AA patients and normal control.

Approach: Sixteen patients with AA and 16 age- and sex-matched healthy controls were enrolled in this study. We analyzed serum levels of reactive oxygen metabolites (ROMs, mainly hydroperoxides, ROOH) using d-ROMs test (FRAS 4, H&D s.r.l., Italy) which represents the level of oxidative stress in the blood. BAP (biological antioxidant power) test (FRAS 4, H&D s.r.l., Italy) was also performed to assess the antioxidant power of the plasma as a measure of the ability to reduce ferric (Fe3+) ions to ferrous (Fe2+) ions.

Results: The mean levels of ROMs in serum of patients with AA (357.69±88.46 CARR U, Carratelli Units; normal value, 250-300 CARR U) were significantly higher than those of controls (287.81±20.22 CARR U, p<0.01). The antioxidant capacity (2063.44±132.67 mmol/l; normal value, 2200-4000 mmol/l) in the serum of patients with AA was significantly lower than that of control (2267.44±135.18 mmol/l, p<0.01).

Conclusions: We could find ROMs were increased and antioxidant capacity was decreased in AA patient. This could support the role of alteration of oxidant-antioxidant enzymatic system in the pathogenesis of AA.

**P-111**

**Intralesional Injection of Cyclosporine A on Patches of Alopecia Areata**

Huh, Chang-Hun;1 Kim, Beom-Joon;2 Youn, Sang-Woong;1 Park, Kyoung-Chan;1
1. Seoul National University, Seoul, Korea; 2. Chung-Ang University, Seoul, Korea

Objectives: Alopecia areata (AA) infrequently manifests a refractory course and we often face difficulties in its treatment. Currently, triamcinolone (TA) intralesional injections (ILI) are regarded as standard treatment option for patch alopecia areata, but continuous ILI of TA may result in skin atrophy. Cyclosporine A (CsA) is an immunosuppressant originally applied in organ recipients, but it is widely used in dermatologic fields including alopecia areata, atopic dermatitis, and psoriasis. We would like to try the CsA ILI treatment for AA, instead of TA-ILI.

Approach: Injectable CsA ample (CIPOL Inj., Chong Kun Dang Pharm., Seoul, Korea) was diluted to 2.5 mg/cc with 5% dextrose solution. Those who have patch AA were selected. And, diluted CsA was injected to the AA lesion every two weeks. Several lesions were treated with TA as a control.

Results: Successful regrowth of hairs could be seen on CsA ILI site without any side effect like skin atrophy. But, the time consumed to induce new vellus hair was quite longer when it compared with that in classical TA-ILI.

Conclusions: We could find CsA ILI also effective treatment for AA patients. And no side effects like skin atrophy can be seen.
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**P-112**

**High Dose Methylprednisolone Therapy on Acute Diffuse Alopecia Areata**

*Hwang, Chul; Back, Seung Ju; Kim, Chang Deok; Im, Myung; Seo, Young Joon; Lee, Jeung Hoon; Park, Jang Kyu; Department of Dermatology, School of Medicine, Chungnam National University, Daejeon, Korea*

Acute diffuse alopecia areata is a unique type of hair loss that mimics anagen effluvium, or in which the initial hair loss is diffuse and followed by total denudation of scalp within several weeks or months. Despite its peculiar clinical feature, the characterization and/or treatment of acute diffuse alopecia areata are very limited currently. In this study, we evaluated the characteristic clinical findings of acute diffuse alopecia areata, and confirmed the effect of high dose methylprednisolone therapy. The medical records of 13 patients with acute diffuse alopecia areata between January 2002 and April 2006 at the Department of Dermatology, Chugnam National University Hospital were reviewed. All patients were treated with high dose methylprednisolone therapy. Of the 13 patients who completed the study, 8 patients (61.5%) were male and 5 (38.5%) were female. The mean age was 29.6 years old. The progression of hair loss was stopped in 2.4 weeks on average from the initial treatment, and newly emerging hairs were recognized in 4.1 weeks on average from the initial treatment. At the end of observation, 84.6% (11/13) of patients showed terminal hair growth and 46.2% (6/13) of patients completely responded to methylprednisolone therapy. In conclusion, we found that acute diffuse alopecia areata can be occurred in male as well as female, and high dose methylprednisolone therapy is the effective method to treat those patients.

**P-113**

**The Cell Infiltration in Alopecia Areata Lesion Is Induced By Th1 Chemokine**

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The current idea towards that alopecia areata (AA) is one of an organ-specific autoimmune disease. Immunocytary cells surround hair follicles in the lesion of acute phase AA, and these cells are constituted by 60-80% CD4+ T cells and 20-40% CD8+ T cells. Th1 cytokine, such as IFN-g, has been dominantly detected in AA-lesion that implicates AA is Th1 disease. Although the phenomenon of cell accumulation in AA-lesion is known very well, the mechanisms of chemotaxis around the HFs has been largely ignored. Therefore, we investigated how the cells accumulate around the HFs in acute AA-lesions. Firstly, the expression of chemokine receptors and chemokines were immunohistochemically investigated. In normal human scalp skin, CXCR3+ and CCR4+ cells were rarely detected, and the number of CCR4+ cells is slightly higher than that of CXCR3+ cells. In contrast, CXCR3+ cells were dominantly accumulated compared to CCR4+ cells in the lesion of AA. In addition, IP-10 was very strongly expressed in and around the hair follicles in AA lesion compared to normal control. Next, in order to investigate the lymphocyte-chemotaxis functionally in AA patients, we used EZ-TAXIScan™ that can show real-time chemotaxis directly under a microscope. PBMC from AA patients showed the strong tendency of chemotaxis to IP-10 compared to TARC. Taken together, it is suggested that increased production of IP-10 may induce accumulation of CXCR3+ T cells in acute AA lesions that results in hair loss.

**P-114**

**Possible Role of p63 and Alpha-6 Integrin in the Hair Loss**

*Kang, Hoon;1 Lee, Ye Jin;2 Lee, Seung Dong;1 Park, Young Min;1 Kim, Hyung Ok;2*

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Objectives: Recent evidence has suggested that p63 plays an important regulator of hair follicle development and increased expression is seen in the outer root sheath. Integrins have a crucial function in the attachment of cells to the extracellular matrix and as important transducers of signals from the extracellular matrix to the matrix cells of the hair follicle. The aim of the current study was to investigate the possibility that p63 and alpha-6 integrin may involved in the pathogenesis of various conditions of hair loss.

**Approach:** Full thickness skin biopsies were obtained from the scalp in each of 15 persons with male pattern androgenic alopecia(MPAA), female pattern alopecia(FPA), alopecia areata(AA), female telogen effluvium(FTE). The control group consisted of 14 persons not having hair loss and any other systemic disease. Immunohistochemical techniques were used to assess p63 and alpha-6 integrin localization. In each section, the percentage of positive cells and the localization of expression were evaluated.

**Results:** In control group, p63 expression was seen in the epidermis and outer root sheath of hair follicles. P63 staining revealed a different pattern in the various conditions of hair loss. In MPAA and FPA, there were weak immunoreactivity in the follicle epithelial structure. In contrast, there were only minor changes of p63 expression in AA and FTE. Among the four hair loss groups, MPAA and AA showed more decreased immunoreactivity
of alpha-6 integrin in the basal sides of follicle epithelium. In control group, all epithelial structure were 100% positive to alph-6 integrin.

**Conclusion:** These expression data suggest a different role of p63 and alpha-6 integrin in the development of hair loss. Since both p63 and alpha-6 integrin are necessary for the hair follicle growth, it is worthwhile to take more concern in this proteins.

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**P-115**

Results of Complex Treatment of Alopecia Areata

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In dermatologic practice Alopecia areata is one of the least studied diseases; it is also of great interest for investigation.

We have singled out one quite interesting case of Alopecia areata from our practice: a female patient, 36 years of age, turned to us in June 2006 with complaints of gradual loss of hair on the head (as focuses), in the groin and the axillary region, on arms and legs, which started in April 2006.

The following examinations were carried out:

- trihogramma
- scalp skin biopsy
- complete blood count;
- coprogramma.
- The diagnosis made:
- Alopecia areata. Lichen planopilaris.
- The treatment given:
  - immunomodulators (sol. Cicoloroni i/m)
  - Bio-Selenium, Anacap;
  - preventive antifungal therapy (tab. Lamizili);
  - external procedures using the infrared laser according to a definite scheme.

**Results:** In 2 months after the beginning of treatment hair growth in the focuses of hair loss has been observed. In 5 months a complete recovery of scalp hair (the hair have become thicker than before) and hair growth in the groin, the auxiliary region, on arms and legs, which started in April 2006.

**Conclusion:** The given case from our practice once again proves the necessity of a more explicit approach to examination of Alopecia areata. It also proves the necessity of use of the complex therapy rather than only the external one.

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**P-116**

Pulse Corticosteroid Therapy For Alopecia Areata: Study of 139 Patients

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**Background/Aim:** Recent reports of pulse corticosteroid therapy for alopecia areata (AA) show its efficacy for patients with a history of one year or less, but not for recalcitrant cases or alopecia totalis/universalis. The purpose of this study was to evaluate the efficacy and safety of pulse corticosteroid therapy for recent-onset AA patients.

**Method:** 139 severe AA patients aged over 15 were included in this study. The duration from the onset of active hair loss was within 12 months for 125 (89.9%) of those patients.

**Results:** 72.7% patients had hair loss on more than 50% of their scalp area. Among the recent-onset (duration of AA ≥6 months) group, 59.4% were good responders (over 75% regrowth of alopecia lesions), while 15.8% with more than 6 months duration showed a good response. Recent-onset AA patients with less severe disease (<50% hair loss) responded at a rate of 88.0%, but only 21.4% of recent-onset patients with 100% hair loss responded. No serious adverse effects were observed.

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**P-117**

Treatment of Severe Alopecia Areata

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Many treatment modalities have been proposed to treat alopecia areata(AA). Of these, systemic corticosteroids and cyclosporine A (CyA) are effective in treatment of severe AA. However, their side effects and high relapse rates have discouraged us from using them in AA. Therefore, combination therapy is required to get adequate efficacy with acceptable toxicity. As CyA and steroids act through different mechanisms, combination therapy with them may produce synergistic effect.

We treated 25 cases of severe AA with combination therapy with systemic corticosteroid (methylprednisolone 16-24mg/day) and CyA (2.5-3mg/kg/day) for 24weeks. In all, 7 patients with alopecia universalis, 6 with alopecia totalis/universalis, 11 with AA involving more than 50% of the scalp and 1 with ophiasis were involved in this regimen. The efficacy of the combination therapy was evaluated at 12 and 24 weeks. We checked peripheral blood concentration of CyA to determine the therapeutic range of CyA with little or no side effects. Satisfactory hair
growth (terminal hair growth occurring more than 50%) was achieved in 68% (n=17), and cosmetically acceptable hair growth (terminal hair growth more than 75%) was in 44% (n=11). In a group which showed satisfactory hair growth, peripheral concentration of CyA was ranged from 30 to 140 ng/ml.

Adverse reactions of therapy were elevated blood pressure in 1 patient, elevated serum cholesterol in 1 patient, which were controlled by medical treatment without cessation of therapy. In these patients, the side effects appeared when the concentration of CyA was elevated more than 200 ng/ml.

This study shows that combination therapy with CyA and low dose corticosteroids would be effective and safe treatment for severe AA, and presents an effective concentration of CyA in peripheral blood.

P-118

Therapeutic Effect of Systemic Cyclosporine in the Patients with Alopecia Areata

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Objective: Cyclosporine is an immunosuppressive agent that has provided new approaches in the treatment of autoimmune disease. The theory of autoimmune pathogenesis of alopecia areata suggests a potential therapeutic effect of cyclosporine. We evaluated the therapeutic effect of oral cyclosporine combined with other modalities in severe and refractory alopecia areata patients.

Approaches: 38 patients (8 patch type alopecia areata, 6 alopecia totalis, and 24 alopecia universalis) were treated with oral cyclosporine 100-200 mg/day with PUVA, DPCP, cryotherapy, intralesional steroid injection, topical steroid and minoxidil solution at least 6 months. Blood pressure, CBC, liver function test, BUN/Cr, electrolyte, and urinalysis were checked to avoid any untoward effects.

Results: Of the 38 patients 23 patients (60.5%) showed new hair growth. 15 patients (39.6%) did not respond to therapy. Mean time until new hair growth was 13.8 weeks. Untoward effects occurred in 9 patients (23.7%), and they were as follows: 5 patients with gastrointestinal discomfort, 2 patients with hypertrichosis, 1 patients with headache and 1 patients with hypertension.

Conclusion: Oral cyclosporine therapy with combined other modalities is recommended in severe and refractory alopecia areata patients.

P-119

A Clinical Study on Alopecia Areata (2001-2006)

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Objective: We performed a study on alopecia areata in order to evaluate the clinical manifestation and compare the efficacies of treatment with intralesional injection of triamcinolone, immunotherapy with diphenylcyclopropenone (DPCP), topical PUVA, cryotherapy and oral cyclosporine.

Approaches: Total 444 patients with alopecia areata including 14 cases of alopecia totalis and 47 cases of alopecia universalis was performed for 6 years from March, 2001 to December, 2006 who visited to the Alopecia Clinic at the Department of Dermatology, College of Medicine, Chung Ang University.

Results: 1) The age distribution showed a peak incidence in the third decade (27.9%) and the mean age was 28.9 years, and 57 cases (12.8%) showed alopecia areata in patients below 10 years old. 2) The most common site was occipital region in both male and female patients; 157 cases (70.0%) and 145 cases (65.9%), respectively. 3) Previous episode of alopecia were observed in 97 (21.8%) and 44 cases (9.9%) had family history. 4) Associated diseases are seborrhoeic dermatitis (35 cases), atopic dermatitis (22 cases), thyroid disease (14 cases), hypertension (13 cases), diabetes mellitus (8 cases), etc. 5) The efficacies of the various modalities had no statistical differences.

Conclusion: These findings suggest that alopecia areata prominently develops in the thirds decade and the efficacies of the various modalities had no statistical differences.

P-120

Expression of Myc/Mad1 in The Human Hair Follicle During Hair Cycle and Alopecia Areata

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Objectives: Precise molecular mechanisms associated with transient growth/differentiation of follicular keratinocyte have not been fully elucidated in the hair cycle. Recent observations have suggested that proto-oncogene c-myc plays a pivotal role in the regulation of both apoptosis and growth in the epithelial cells. Mad1 is a Myc antagonist that functions as a transcriptional repressor. Inhibition of proliferative activity subsequent to Mad1 overexpression has been demonstrated in a variety of cell types, including...
keratinocytes. However, little is known about the Myc/Mad1 expression in follicular keratinocyte. To elucidate whether c-Myc/Mad1 proto-oncogenes could be involved in normal human hair cycle and alopecia areata, in situ expression of Myc/ Mad1 was investigated.

Methods: Immunohistochemical analysis were performed for the specimens taken from surgery of patients and from patients of alopecia areata. Antibodies against PCNA (Proliferating Cell Nuclear Antigen), ki67, c-Myc and Mad1 were used.

Results: Immunoreactivity for ki67 was observed in the bottom portion of inner root sheath in human anagen hair follicle. In contrast, c-Myc-positive cells were visible in outer root sheath cells of bulge area. We compared expression of c-Myc/ Mad1 in normal human hair follicles during hair cycle and hair follicles of alopecia areata.

Conclusions: c-myc and mad overexpression might be related with switching to the hair cycle. These findings suggest that c-myc has been implicated not only in the cell proliferation, but also in the terminal differentiation and mad has been implicated in the cell differentiation in hair cycle.

P-121
Anthralin For Alopecia Areata:
A Half-Side Comparison

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Objectives: Anthralin application is an option for topical immunosuppressant treatment of alopecia areata (AA). No controlled trials have been done. We report the successful treatment of AA by anthralin as demonstrated by half-side comparison.

Approach: Seven patients, 5 female, 2 male, aged 12 to 52 years (median 36 years) suffering from almost total AA or AA totalis for at least 2 years were treated daily with 1 or 3% anthralin cream on one half of the scalp only. In addition, 5% minoxidil solution was applied to the entire scalp twice daily. Skin biopsies for pathohistology were compared from anthralin-treated and non-treated sites. Scap photographs were also taken.

Results: In the 7 patients treated, hair growth on the anthralin-treated half of the scalp set in around 5 months and continued to increase up to 18 months. After definite hair growth on the treated side, anthralin was also applied to the other half of the scalp. No side effects other than slight burning and brownish discoloration were noted. In scalp biopsies from treated areas exhibiting regrowth of hair, the lymphocytic infiltrates surrounding hair follicles tended to vanish, and the scalp tissue normalized, there were no signs of allergic contact dermatitis.

Conclusion: Our results indicate that topical immunosuppressant treatment of alopecia areata for 5 to 18 months with anthralin combined with topical minoxidil is a safe and efficacious treatment associated with normalization of scalp tissue.

P-122
Acute Diffuse and Total Alopecia

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Alopecia areata (AA) commonly starts with ovoid patches of hair loss and then presents several different clinical forms. The prognosis of AA is unpredictable. Indicators of a poor prognosis are long duration, family history of alopecia, and extensive hair loss. Alopecia totalis is well known to have a poor prognosis, so it is commonly resistant to several treatments and frequently recurs. We studied 23 patients(21 female and 2 male) with AA who showed extensive hair loss. But they present cosmetically acceptable hair regrowth within 6 months. Most of them were treated with DPCP, but some of them didn’t have any treatment. They had a very acute course of total hair loss within two months. They don’t have a history of systemic disease, or previous history of alopecia areata. Most patients were female in around thirty. The histopathology of the lesion revealed an infiltration of mononuclear cells around the hair follicles. These above findings indicate that these cases should be categorized as a subtype of AA, “acute diffuse and total alopecia”, which shows favorable prognosis.

P-123
Perinevoid Alopecia – a Distinct Entity or Variant of Alopecia Areata?

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Alopecia occurring around a naevocellular nevus or Perinevoid alopecia, since its original description in the German literature in 1958 has rarely been reported subsequently. The senior author Prof. Patrick Yesudian published 3 cases in 1976 and also suggested that perinevoid alopecia is a distinct entity and not merely a variant of alopecia areata.

We report 6 further cases of perinevoid alopecia. In all these cases a central naevocellular nevus of varying duration was surrounded by an area of alopecia which was the presenting complaint. The excised central mole showed histopathologically intense lymphocytic infiltrate pervading nevus cells of varying numbers. Mucin deposition in the...
dermis was demonstrated with special alcian blue stain. No regrowth of hairs was seen in the patch of alopecia after removal of the central on several months follow-up.

We present these cases for their rarity and review the meagre literature on this entity.

P-124
Analytical Versus Empirical Drug Screening Approaches For Alopecia Areata

Hall, Leon;1 Bunker, Elizabeth;1 Sun, Jing;2 Silva, Kathleen A.;2 Szatkiewicz, Jin P.;2 King, Benjamin L.;2 Kamdar, Sonya;2 King, Lloyd E.;2 Sundberg, John P.;2
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While numerous drugs continue to be used to treat patients with alopecia areata (AA) most patients are disappointed with the results. Treatments continue to be devised empirically and tested in small trials. The C3H/HeJ mouse skin graft or spontaneous models for AA provide tools for screening individual and combinations of drugs for efficacy and safety. More importantly, we can now move from an empirical to an analytical approach. Using the Affymetrix(r) gene expression tools (GeneChip Mouse Genome 430 2.0 Array) with the Ingenuity Pathways Analysis(r) software we evaluated mice at 5, 10, 15, and 20 weeks after skin grafting and mice with spontaneous AA compared to age and gender matched controls. Gene networks were identified but more importantly, FDA-approved drug targets for dysregulated gene products were defined. These data provide tools to both prioritize drug treatment options and test them for efficacy and safety. C3H/HeJ mice with AA can be treated by topical, local, systemic, or oral routes with a comprehensive analysis done to validate efficacy. Information on accessing the models for setting up drug studies can be found at http://jaxmice.jax.org/services/aloepecia_areata.html and http://jaxmice.jax.org/library/notes/504/504b.html.

P-125
Funding Opportunities for Alopecia Areata Research Through the National Alopecia Areata Foundation

Kalabokes, Victoria;1 Norris, David;2 Shapiro, Jerry;2 Bystyn, Jean-claude;2 Christiano, Angela;2 Duvic, Madeleine;2 Gordon, Gary;2 McMichael, Amy;2 King, Lloyd E.;2 Sundberg, John P.;10
1. National Alopecia Areata Foundation, San Rafael, CA, USA; 2. UCHSC at fitzsimons, Denver, CO, USA; 3. University of British Columbia Skin Care Centre, Vancouver, BC, Canada; 4. NYU School of Medicine, New York, NY, USA; 5. Columbia University, New York, NY, USA; 6. MD Anderson Cancer Center, Houston, TX, USA; 7. National Alopecia Areata Foundation, Highland Park, IL, USA; 8. Wake Forest University School of Medicine, Winston-Salem, NC, USA; 9. Vanderbilt University, Nashville, TN, USA; 10. The Jackson Laboratory, Bar Harbor, ME, USA

For the past 20 years, the National Alopecia Areata Foundation (NAAF) funded over 135 broad based research projects ranging from epidemiology, clinical trials, fundamental immunology, animal models, to genetics. These pilot and feasibility projects help investigators generate preliminary data necessary to apply for and obtain funding from major granting agencies. This year NAAF awarded 7 new research grants. These one year research grants are awarded for up to $50,000 (USD) and can be reapplied for in subsequent years. Numerous publications are the direct result of these grants which focused and propelled research in this area forward. This year we initiated a fellowship for 2 years to support a postdoctoral investigator to help them build a career in alopecia areata research. Future requests for applications will give priority to projects dealing with immunology of alopecia areata. Details on these grant opportunities and access to applications and instructions can be found at http://www.naaf.org/research/research-grantapplication2006-2007.asp.

P-126
Large Scale Immune Response Gene Expression Analysis Defines Alopecia Mouse Models

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Identifying the correct mouse model for psoriasis, alopecia areata, cicatrical alopecia, and other inflammatory and noninflammatory skin and hair diseases is a constant debate. Integrating large scale quantitative real time RT PCR (qPCR), histopathologic, and cytologic approaches, we evaluated spontaneous alopecia areata in C3H/HeJ mice, a form of psoriasiform dermatitis in chronic proliferative dermatitis (cpdm/cpdm) mutant mice, and a form of cicatrical alopecia in C57BL/6J mice. Our 384 qPCR set of immunoregulatory genes rapidly provided an easily interpretable profile for each model that was unique. The alopecia areata model results confirmed many of our previous studies indicating this is a T cell (primarily CD8+) driven disease. A longitudinal Affymetrix array experiment using the alopecia areata graft induced model not only confirmed these results but demonstrated a progressive and significant increase in expression of Cd8a
but also a series of genes in the canonical pathway. By contrast, chronic proliferative dermatitis is an inflammatory proliferative skin disease with a predominant eosinophil component. Changes were unique and reflected the pathogenesis of the mutant inflammatory skin disease phenotype. Early stage cicatrical alopecia had minor expression changes indicative of a nonimmunologic pathogenesis. Expression changes were consistent with histopathology and immunofluorescence results. This proof of concept approach provides the prototype for large-scale implementation to carefully define immunological mechanisms of skin diseases in mouse models and through translation, new molecular tools to accurately define human inflammatory skin diseases.

P-127
Deficiencies in the Paracrine Signalling System of Stem Cell Factor (Scf) and Its Receptor, C-Kit, Occur in Human Hair Greying

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Loss of hair pigmentation is a poorly understood aging phenomenon. Stem cell factor (SCF) regulates rodent pigmentation via c-kit. We showed previously that cultured human dermal papilla cells secrete SCF suggesting dermal papillae as local SCF sources. To determine whether alterations in the SCF/c-kit signalling system are involved in human greying, we investigated the expression of scf and c-kit genes in pigmented and non-pigmented hair follicles using semi-quantitative RT-PCR and located melanocyte antigens and c-kit by immunohistochemistry of cryosections.

Lower parts of anagen follicles were microdissected from human scalp samples treated with RNAlater to inhibit mRNA degradation. Total and poly(A)RNA were isolated, cDNA prepared and RT-PCR carried out in a dose responsive manner using primers for c-kit, SCF and control ß-actin.

Pigmented follicles (n=5) expressed c-kit and both soluble and membrane-bound scf. Immunohistochemistry located melanocytes exhibiting c-kit and melanocyte antigens in the hair bulb and outer root sheath. In non-pigmented follicles (n=5) expression of all three genes and melanocyte numbers in the bulb were significantly reduced.

The expression of scf and c-kit within human follicles and the presence of c-kit on melanocytes strongly support a paracrine role for this signalling system in human hair pigmentation. Soluble SCF would correspond to that secreted by dermal papilla cells, presumably influencing bulb melanocytes; membrane-bound scf may be involved in melanocyte migration. The reduced expression of scf and c-kit implies that failure of this system causes human greying. This may lead to new treatments; as cells still express c-kit, exogenous SCF may facilitate re-pigmentation.

P-128
A Retrospective Clinical Study of Alopecia Areata in Canada (2001-2004)

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Objectives: To evaluate the clinical manifestations and treatment of AA in a mixed ethnic population.

Approach: Alopecia Areata (AA) is a common disease of patchy non-scarring hair loss. There are 400-700 patients with AA in our study. Up to this point, we have tabulated the results of 100 patients.

Results: The ratio of males to females was 1:1.38 with 63 Caucasians, 19 Orientals, 9 East Indian, 8 Middle Eastern and 1 African-Canadian. The mean age on initial visit was 42.27 years. The family history was contributory in 12 cases. The majority of patients had AA of 3-12 months duration. The relapse rate was 15%. The most common site of predilection was the occipital area. Associated with diseases were: atopy, vitiligo, psoriasis and thyroid problems. The success rates of intralesional corticosteroid was 90% in limited AA, and DPCP immunotherapy for patients with alopecia totalis, universalis and ophiasis was with success rates of 78%.

Conclusion: For limited AA, intralesional corticosteroid was the first line treatment, as well as DPCP immunotherapy was the best option for patients with alopecia totalis, universalis and ophiasis.

P-129
Topical Immunotherapy With Diphenylcyclopropenone For Treatment of Alopecia Areata in Taiwan

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Background: The response rate of topical immunotherapy with diphenylcyclopropenone (DPCP) in the treatment of alopecia areata (AA) varied from 4-85% in the literature. This study was aimed to evaluate its efficacy in local Taiwanese patients.
Methods: Twenty patients with extensive AA were enrolled in this study, including 6 males and 14 females (age range 6-52 years, mean age 21.4) with disease duration between 6 months and 40 years (mean 10.3 years). DPCP was applied at 2-week interval and hair regrowth was evaluated after 6-month therapy.

Results: Twelve of 20 patients have completed 6-month therapy, while five are still undergoing treatment and two are dropped out due to intolerance. Hair regrowth more than 50% was observed in six patients (50%), in whom three had complete response (90-100% terminal hair regrowth) and the other three had partial response (50-90% terminal hair regrowth). The remaining six patients (50%) had no significant hair regrowth. There was no correlation between the response and the age of onset or disease duration (P=0.4 and 0.2, respectively). Two patients with pitting nails were not responsive to DPCP treatment. Side effects included pruritus 85% (17/20), blistering 50% (10/20), auto-sensitization 40% (8/20), occipital lymphadenopathy 20% (4/20) and hyperpigmentation 10% (2/20). Partial recurrence was observed in 33% (2/6) of responders in 2-26 months of follow-up after cessation of treatment. Among eight patients with AA totalis/universalis, five (62.5%) presented hair regrowth more than 50%.

Conclusion: DPCP topical immunotherapy seems to be effective for extensive AA patients, especially those with AA totalis/universalis. The most common side effect is pruritus. Recurrence after hair regrowth still poses a problem.

P-130
Alopecia Totalis With a Good Prognosis In a Patient Receiving Transient Clomifene

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Objectives: Alopecia areata is an autoimmune hair loss disorder characterized by peribulbar infiltrate of T cells and hair cycle shift. Estrogen is well-known to modify immunological states and extend the anagen phase. To elucidate alopecia areata etiology,

Approach: Description of the clinical and histological findings in a patient with alopecia totalis. A 34-year-old woman with second infertility undergoing oral clomifene developed sudden total hair loss 3-weeks after first interval of taking clomifene. A biopsy of the scalp was transversely and horizontally sectioned.

Results: Transverse sections showed peribulbar infiltrate, consistent with the diagnosis as alopecia areata. Horizontal section showed a decreased number of anagen hair follicles and a increased number of regression hair follicles. Soon after the hair shedding, terminal hair regrowth occurred.

Conclusion: Our patient developed alopecia totalis with a good prognosis after transient estrogen blockade by clomifene, which may suggest the hormonal role in the etiology of alopecia areata. Sudden estrogen blockade may have effect on hair cycles or immunological states or both, which might lead to trigger the onset of alopecia areata.

P-131
Lipodystrophia Centrifugalis of the Scalp Presenting With Arch-Form Alopecia: A 9-Year Follow-Up Observation

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A 2.5-year-old boy developed slightly depressed lesions with light erythematosus border on the right side of the neck and chin measuring 45mmx70mm and 35mmx38mm, respectively. SS-A/SS-B related erythema, LE profundus, and erythema annulaire centrifugum were suspected, but no lines of evidence supporting the diagnosis were obtained. When he was 5.5 years old, at second visit, right chin lesion had disappeared but right neck lesion had extended centrifugally partly into the scalp, and linear arch-form alopecia was apparent on the right temporal region along with the edge of erythema. Hair regrowth was seen inside of the slightly depressed lesion. Two pea-sized regional lymphnodes were palpable. Biopsies taken at the alopecia showed non specific, non pilotropic lymphocytic inflammation in the subcutaneous fat. MRI findings revealed loss of subcutaneous fat inside of the lesion and helped to make diagnosis of the case as lipodystrophia centrifugalis developed on the neck and scalp. The alopecia continuously extended until he became 11 years old resulting in large arch-form alopecia Thereafter, expansion ceased and hair regrowth occurred in the linear alopecia. The 9-year follow-up of this case clearly demonstrates that centrifugal lipodystrophy might involve the scalp and cause linear arch-form alopecia.

P-132
Unusual Forms of Scarring Alopecia in Women – Peripheral Centripedal Scarring Alopecia?

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We experienced several cases of unusual forms of scarring alopecia in women. Case 1 was 45-year-old woman. Seven years ago, hair loss started in left temporal area and then spreaded to all the marginal areas of scalp. Her hairs in eyebrow were intact and there were no
evidence of hyperandrogenemia. Case 2 was 24-year-old female. Hair loss began in the frontal area at the age of 12, and then nearly all the margins of scalp became involved. She had regular menstruation and no evidence of hyperandrogenemia. Her eyebrow hairs were very sparse. Case 3 was 64-year-old woman. Six months ago, hair loss was observed in both frontal areas. She was postmenopause and had no evidence of hyperandrogenemia. Her brow hairs were intact. In the histopathologic examinations of these cases, no evidence of alopecia areata or lichenoid inflammation were noted. They were compatible with burn-out alopecia. With only clinical finding of these patient, many diseases should be considered as possible diagnoses. In non-scarring alopecia, alopecia areata and female pattern hair loss (Hamilton type) were possible candidates. In Scarring alopecia, there were fibrosing alopecia in patterned distribution, pseudopelade and frontal fibrosing alopecia. But, our cases were not compatible with these in clinical and histopathological features. And so, we think these cases can be a new disease entity of scarring alopecia and propose ‘peripheral centripetal scarring alopecia in women’ for these cases. We expect more cases to be collected and analysed for the validation of this new entity.

P-133
Hair Loss in African American Women with Focus on Central Centrifugal Cicatricial Alopecia

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Central centrifugal cicatricial alopecia (CCCA) is the term given to the destructive hair loss over the central scalp seen primarily in African American women. It is the most common type of hair loss in African American women and usually presents to dermatologists when scarring is obvious and little can be done to reverse the hair loss.

In an attempt to better understand the prevalence of this condition and the factors that may be involved in the initiation or accentuation of the process, members of the North American Hair Research Society (NAHRS), with the support of Procter and Gamble, have developed a photographic scale to rate the degree of hair loss in CCCA and have begun a project to assess the level of agreement between patient and dermatologist in using this scale. In addition, the team has piloted and begun the validation process of a questionnaire assessing multiple familial, environmental, hair grooming and medical factors that could be implicated in this condition. Results from our first screening of ~150 women will be presented.

P-134
2 Cases of Folliculitis Decalvans after Hair Restoration Surgery

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Objectives: We report 2 cases of folliculitis decalvans in patients who underwent hair restoration surgery.

Case 1: 43 year old male patient developed severe pain, itching, papules, pustules, crusts and follicular tufting in the area of the grafts, 22 years after his hair transplant surgery.

Case 2: 47 year old patient with MPHL showed a hairless patch on the crown in 1992. The biopsy confirmed MPHL and showed no signs of inflammation or scarring. The patient underwent hair transplant surgery in January 1993 for the restoration of his hair line. 8 weeks after the procedure the patient showed erythematous papules and pustules around the hairless area on the crown.

Approach: A 4 mm punch biopsy was taken from the affected area.

Results: The pathology results showed folliculitis decalvans in both cases.

Case 1: Treatment: Minocin 100 mg twice daily and topical fucidic acid and Hydrocortisone (Fucidin H(r) cream). The lesion improved greatly after 4 weeks.

Case 2: Treatment: Cloxacillin 500 mg 4 times a day for 2 weeks, topical fucidic acid (Fucidin(r) ointment). The condition improved and the patient underwent a second session of hair transplant surgery in 1996. 2 years after the second surgery he showed signs of active folliculitis decalvans. Treatment: Minocin 100 mg twice daily and Fucidin(r) cream. The lesion stabilized over the following 2 years.

Conclusion: Folliculitis decalvans can be aggravated by surgical procedures such as hair transplantation. A thorough scalp examination and discussion of risks is crucial before surgical scalp procedures are performed.
**P-135**

**Lupus Erythematosus Associated Alopecia: Cleveland Clinic Experience on Diagnosis, Evaluation and Treatment**

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**Background:** Alopecia is a common feature of discoid lupus erythematosus. There is currently few data in literature that delineates the varied presentations of hair loss in lupus erythematosus.

**Objective:** To evaluate the clinico-pathological correlation of lupus erythematosus and its associated alopecia in attempts to standardize the diagnosis and effective treatment of the disease.

**Methods:** We reviewed the medical records and biopsy results of 19 patients with lupus erythematosus and alopecia that were seen in the Cleveland Clinic Department of Dermatology between 2003 and 2006.

**Results:** The most common clinical presentation was multiple patchy plaques due to lupus erythematosus. Disease progression was halted and improvement of active lesions was seen in patients on Plaquenil (anti-malarial) and intralesional Kenalog (steroid).

**Limitations:** This study was limited by being retrospective in nature.

**Conclusion:** Clinical presentation of hair loss due to lupus erythematosus varies with degree of disease involvement and disease state at time of clinical presentation. The most common clinical finding was the presence of multiple plaques in the scalp. The most frequent histopathological finding was lymphocytic infiltrate. While previous data has showed that anti-inflammatorieties are efficacious in the treatment of lupus associated alopecia, our incidental and anecdotal finding supports the combined use of steroids and anti-malarials.

**P-136**

**A Case Report of Hair Regrowth in Central Centrifugal Cicatricial Alopecia With Topical Tacrolimus 0.1% Ointment**

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**Background:** Central centrifugal cicatricial alopecia (CCCA) is an insidious form of primary lymphocytic scarring alopecia of the crown that occurs predominantly in adult black women. Most attribute the condition to the repeated use of traumatic hair grooming practices. Current recommendations for management are the cessation of this behavior, the adoption of natural coiffures, and treatment with topical and/or intralesional corticosteroids, and tetracycline adjunctively. In the authors’ estimation, results are often disappointing. Camouflage remains the best option.

**Objective:** To determine if topical tacrolimus, a modulator of T-lymphocyte activation with trichogenic properties in some humans and in rodent models, has therapeutic value in CCCA.

**Approach:** Two subjects with advancing disease and limited to no response to conventional treatment were prescribed 0.1% topical tacrolimus ointment twice daily. Treatment response was based on subject and dual clinician global assessment of hair regrowth and scalp coverage, and was followed with serial photography.

**Results:** Two female black subjects, ages 54 and 73 years, with progressive longstanding disease, were treated for 36 and 25 months, respectively. By all measurements, one subject demonstrated hair regrowth after 6 months of treatment, with noticeably improved scalp coverage over the ensuing years of treatment. The other subject reported hair regrowth between months 4 to 18, with stabilization thereafter; this finding was not substantiated by clinical or photograph review.

**Conclusion:** Topical tacrolimus holds great promise in the management of some subjects with CCCA. In-depth characterization of treatment response determinants along with a formal, half-head study of adequate sample size are needed to further explore and quantify this therapeutic potential.

**P-137**

**Cicatricial Pemphigoid Involves the Scalp With Scarring Alopecia**

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**Objectives:** To discuss scalp involvement in cicatricial pemphigoid.

**Approach:** A 56 year old female presented with scalp and oral findings for one year. Her scalp was extremely itchy, burning and painful. She also presented with pain in the mouth. There is also a history of joint pain and general malaise. There were some eye symptoms. Physical
examination revealed scarring hair loss with erosions. Erosions were also present in the oral cavity. But there was no abnormality on eyes.

**Results:** Histopathology and immunohistopathology was consistent with cicatricial pemphigoid. Patient was treated successfully after 3 months of dapsone and topical superpotent corticosteroid ointment. Symptoms and physical findings improved markedly.

**Conclusion:** Cicatricial pemphigoid may present as a secondary scarring alopecia and can be treated successfully with oral dapsone and superpotent topical corticosteroids.

**P-138**

Monilethrix In Tasmania

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Monilethrix is a rare autosomal dominant hair shaft dysplasia that results in beaded hair fibres that are fragile. Affected individuals may present with alopecia, keratosis pilaris and rarely nail dystrophy. Clinically, the monilethrix phenotype is variable although penetrance is usually complete. To date, eleven mutations in three type II hair cortex keratins have been identified as responsible for dominant monilethrix. Recently, mutations in desmoglein 4 (a desmosomal cadherin) have been found to cause rare cases of recessive monilethrix.

We identified a large multigenerational Tasmanian kindred that demonstrates autosomal dominant monilethrix with variable penetrance. We undertook a clinical-genetic study of this family, comparing clinical findings with hair microscopy and hair fibre tensile strength.

We contacted 330 family members. We examined 119, and obtained 3 hair samples (from different scalp sites) from each. There were 18 affected individuals and 101 unaffected. Of those affected: 5 individuals were severe, 8 were moderate and 5 were mildly affected. Of those clinically unaffected: 3 provided a history of childhood monilethrix and 15 were obligate carriers, having transmitted the disorder to their children.

There was a wide spectrum of hair microscopic findings, which could not be consistently related to clinical severity. At this stage, hair fibre tensile strength studies do not show a consistent relationship with clinical severity. There were significant intra-patient differences on hair microscopic examination.

Our study describes a large monilethrix family displaying incomplete penetrance and a wide spectrum of clinical severity with discordant microscopic findings.

**P-139**

Familial Clouston Syndrome

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Brother and sister presented with total hair loss, affecting the scalp and eyebrows, dating since birth. A second brother presented with scanty scalp hair. Father was similarly affected. There was no history of parent consanguinity. Grandmother and cousins had only scanty scalp hair.

The cases presented also with peculiar papular pebbling on the ventral and dorsal aspects of the fingers. On the palmer side, the papules were arranged along the finger print lines. On the dorsal surface, the involvement of the nail bed led to near complete destruction of the nail plates. The younger sister presented with peculiar notching along the biting edge of the incisors, simulating the pebbling on the surface of the skin.

**P-140**

Hypotrichosis Simplex – A South Indian Variant?

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In our tropical hair clinic at Chennai formerly Madras considerable number of patients present with congenital hypotrichosis. There is no other congenital abnormality apart from hypotrichosis. It does not fit in any of the previously listed congenital hypotrichoses. We present few of this mysterious hypotrichosis which could be sporadic mutations.

Patients of all ages varying from 2 years to 35 years present with hypotrichosis of the scalp hair often existing from birth or after the first tonsure. The other appendages are normal except for trivial dental abnormalities. The hair is thin, dry, brittle, lustreless and fragile. The light microscopy does not reveal any abnormality. We lack facilities to do electron microscopic studies. Majority of the cases are sporadic and lack family history. Most of them are born to consanguinous parents.

It is very difficult to classify and type the congenital alopecia without genetic studies. There is some response to topical minoxidil.
Poster Abstracts

It appears that these cases could represent a particular south Indian variant of Hypotrichosis simplex. We seek help from the sophisticated centres for genetic study.

P-141
Genomic Analyses of Two Japanese Pedigrees with Monilethrix
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Monilethrix is an autosomal dominant hair disorder characterized by the beaded appearance of the hair due to periodic thinning of the shaft. This disorder is reportedly caused by mutations in the helix termination motif of two type II cortex keratins, hHb1 and hHb6. We analyzed the genomic sequences of these keratins for two independent Japanese pedigrees with Monilethrix, a 32-year-old mother and her 3-year-old daughter, and a 27-year-old woman and her 5-year-old son. We detected a heterozygous point mutation of E413K (change of GAG to AAG at codon 413) in exon 7 of the hHb6 gene in both pedigrees. Scanning electron microscopy revealed a constricted internodal alternating pattern in the hair samples of these patients. Topical application of minoxidil and careful treatment of the hair were effective treatments in these cases. Genotype/phenotype correlation was not obvious in our cases or in the previously reported cases harboring this mutation.

P-142
Successful Treatment of Temporal Triangular Alopecia By Hair Transplantation
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Objectives: To determine if hair transplantation is a viable treatment for temporal triangular alopecia (TTA).

Approach: Temporal triangular alopecia is a form of non-scarring alopecia on the temporal scalp. It occurs very rarely and appears usually in children at birth up to 6 years of age. It is often unresponsive to any medical treatment. We present a case of a 17-year-old boy with a 3.5 x 2 cm patch of TTA since birth on the left temporal portion of the scalp. Hair transplantation was performed by removing a 3.5 x 1.05 cm strip from the occipital donor area and subdivided into 325 follicular units. These were placed with dense packing into the recipient area of TTA.

Results: A cosmetically successful result was seen 16 month post-operatively.

Conclusion: Hair transplantation is a successful option for patients with TTA.

P-143
Epilatory Effect of Glycyrrhizic Acid
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Hypertrichosis, hirsutism and giant hairy nevus are well known examples of abnormal hair growth with some risk of a significant negative impact on the psychosocial development of affected people. So far, all known methods for hair removal are more or less effective and show partly considerable side effects like pain, skin irritation, contact eczema, folliculitis, and hyper-pigmentation. In co-operation with a study group of Turkmenistan we found a new principle of painless and rapid hair removal based on liquorice, a commonly used herbal extract of the traditional Asian medicine. In the meantime we defined the liquorice compound glycyrrhizic acid to be responsible for the epilatory effect. We dissolved 15% glycyrrhizic acid in an aqueous solution containing 10% urea and 20% ethanol and treated wistar rats in the neck region twice a day. After 3 days first indications for hair loss became visible. After 6-12 days the treated skin was nearly free of hairs without any sign of skin irritation. Even after a periodically long term treatment over one year no abnormality of the skin surface was visible, but a permanent reduction in re-growing hair quantity by more than 50%. Based on these findings Glycyrrhizic acid is a candidate molecule for the development of a powerful agent for painless and permanent hair removal.

P-144
Dandruff Is Characterized By Increased Cytokines in the Stratum Corneum
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Dandruff is a condition that is characterized by the presence of flakes on the scalp and/or itch. This condition is known to be associated with decreased levels of scalp skin ceramides, similar to those found in dry skin.

In this study, we investigated the levels of inflammatory markers in the scalp skin of Asian panelists. Panelists were recruited and segmented into healthy (no visual flakes) and dandruff (flakes adhering to scalp skin) groups. Stratum
corneum samples were collected and extracted. These samples were analyzed for cytokines by ELISA and total protein by Bradford assay. Extractable protein increased in the dandruff group compared with healthy subjects, most likely as a result of the increase in adherent flakes. Cytokines were expressed as pg cytokine/ug protein. The ratio of interleukin-1a receptor antagonist protein (IL-1RA) to IL-1a was found to increase dramatically from 20:1 in healthy scalp panelists to over 100:1 in those with dandruff (P < 0.01). Likewise, IL-18 was also found to increase dramatically from 0 to ~500 pg/ug protein in the two groups respectively (P < 0.01). Prostaglandin E2 was detected, but no changes were seen between groups. Tumor necrosis factor-a, IL-1b, IL-6, and IL-8 were not detectable in the samples. These data demonstrate that dandruff is associated with perturbations in inflammatory markers in scalp skin stratum corneum. This confirms previous hypotheses suggesting that dandruff is driven by a number of factors, including the quality of the stratum corneum and its interaction with Malassezia.

P-146

The D.H.I.® Technique – A Smart Quality Control System – A New Method of Hair Restoration

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The D.H.I.® (Direct Hair Implantation) technique, is a minimally invasive method of hair transplantation which avoids the need for a strip of donor skin to be removed from the back of the head. Therefore no scalpels or stitching are required at any point during the procedure. Single hair follicles are extracted one by one from the donor area (back of the head) and re-implanted with the unique patented device, the DHI Implanter.

The method Direct Hair Implantation(r) guarantees 100% natural results and maximum survival of implanted hair follicles through a minimally invasive procedure with no visible scars. The DHI Medical Group developed a new Protocol to overcome the negative aspects of the strip procedure, such as: scarring, patients’ fear of surgery, donor area follicle destruction, uncontrolled placement that created unnatural results and poor growth. DHI comprises the largest international medical and research medical team exclusively involved in the treatment of scalp and hair disorders. DHI developed and patented the new method that have gained the recognition of the world medical community. The crowning achievement of our life time commitment is the direct hair implantation medical procedure, also knowing as simply the D.H.I.® Technique. The D.H.I. Technique has now been in existence for over 37 years and thousands from around the globe enjoy the amazing results.

DHI is simply the best solution to all scalp and hair disorders.

P-148

A Comparative Clinical Trial of Evaluation of the Efficacy of Oral Terbinafine (Lamisil) and Prednisolon in Patients With Seborrheic Dermatitis

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Background: Previous trials have suggested that oral terbinafine, , could be useful in the treatment of seborrhoeic dermatitis.

Objectives: To investigate the clinical efficacy of oral terbinafine , in patients with moderate to severe seborrhoeic dermatitis.

Methods: fifty outpatients (19 men and 31 women) with moderate to severe seborrhoeic dermatitis were enrolled in the study. After a 2-week wash-out period, patients were randomized to either oral terbinafine (n = 25 or prednisolon (n = 25) daily for 4 weeks . Patients were followed up for an additional 8 weeks . They were evaluated at weeks 0, 2, 4 and 12 . The end-point of the study was clinical evaluation of erythema, scaling and itching, on a 0-3 scale. A global clinical score, was also calculated.

Results: At baseline, the mean ± SD global clinical score was 6.68 ± 1.8 in the prednisolon group and 6.56 ± 1.89 in the terbinafine-group. At weeks 1 the mean ± SD global clinical score in the prednisolon group was 3.36 ± 1.75 and 4.56 ± 1.73. At weeks 4 the mean ± SD global clinical score in the prednisolon group was 2.48 ± 1.53 and 3.18 ± 2.35, At week 8 the mean ± SD global clinical score in the prednisolon group was 2.64 ± 1.73 and 4.28 ± 2.41,respectively, which was significantly different from baseline. As compared with baseline values and the prednisolon group, terbinafine treatment (P = 0.82, T-test) reduced the mean ± SD global clinical score. No serious adverse events were recorded in either group.

Conclusions: This is the first trial to show oral terbinafine being effective in the treatment of moderate to severe seborrhoeic dermatitis.
P-149
Ebastine, a Second-Generation Antihistamine, as a Supportive Medication For Alopecia Areata

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Despite our accelerated understanding of the pathophysiology of alopecia areata (AA), evidence-based remedies for AA are still limited. Thus, there are clear demands on new therapeutic approaches. A couple of clinical studies in the Japanese literature reported favorable effects of second-generation antihistamines, including ebastine, for the treatment of AA. In addition, we also experienced promoted hair regrowth in some AA patients after oral administration of ebastine. To objectively assess the efficacy of ebastine for the treatment of AA, we performed open-labeled study using C3H/HeJ AA model mice. Seven pairs of C3H/HeJ littermate mice with similar extent of AA lesions were prepared. One of each pair of mice orally received ebastine (1.5mg/head/day) daily for 4 weeks, while another mouse was given control solution. About 2 weeks after the initiation of the trial, apparent hair regrowth in AA lesions was observed in 3 out of 7 ebastine-treated mice. In contrast, no improvement of AA lesions was noticed in placebo-treated mice. Histopathological investigation of AA affected sites of placebo-control mice revealed intensive inflammatory cell infiltration around anagen hair follicles and moderate cell infiltration within subcutaneous fat layer beneath telogen hair follicles, while such cell infiltration was noticeably reduced in AA sites of ebastine-treated mice, irrespective of phenotypical improvement of hair loss. Further investigation is necessary to definitively address the effect of ebastine in human AA, however, these findings, together with our clinical observation, suggested that ebastine could be a useful supportive medication for AA.

P-150
The Efficacy and Safety of AP-FHG0604T on Female Pattern Hair Loss

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Background: Many anti-androgen drugs, mineral supplements and topical minoxidil have been used for the treatment of female pattern hair loss (FPHL). However they do not always achieve the successful results and there is still much desire for more effective therapy.

Objectives: The purpose of this 18-week, double-blind, placebo-controlled, randomized clinical trial was to investigate the efficacy and safety of a new topical agent, AP-FHG0604T in the treatment of FPHL.

Approach: A total of 33 women (mean age: 33.4 years old) with FPHL applied topical AP-FHG0604T solution (n = 17), or placebo (vehicle for AP-FHG0604T solution; n = 16) twice daily. Efficacy was evaluated by phototrichogram, investigator's photographic and patient's subjective assessments. All adverse effects during the study were reported.

Results: After 18 weeks of therapy, topical AP-FHG0604T treatment showed significant improvement compared with baseline values in total hair count, non-vellus hair count, and linear hair growth rate. In the placebo group, non-vellus hair count and ratio of anagen hair significantly decreased. The change rates of total hair count and non-vellus hair count in AP-FHG0604T group were significantly higher than those in the placebo group. Neither investigator's photographic assessments nor patient's subjective assessments of hair growth showed statistically significant differences between AP-FHG0604T group and placebo group. Some patients who used AP-FHG0604T complained of local irritation during the study, but the irritation was so mild that they did not need any treatments for it.

Conclusions: We conclude that AP-FHG0604T is a safe and efficient topical agent that can be another treatment of choice for FPHL, as shown by objective assessment with phototrichogram.

P-151
How Does Minoxidil Stimulate Hair Growth? – A Mechanism Via Potassium Channels in Human Hair Follicles

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How minoxidil acts is currently unclear, despite widespread topical use for hair loss. Suggested mechanisms include vasculature stimulation. Other potassium channel opening drugs (PCOs) also stimulate hair growth implicating ATP-sensitive potassium (K_{ATP}) channels. We recently demonstrated that minoxidil and other PCOs stimulated deer follicle growth in vitro (Davies et al., 2005), but human studies are inconclusive.

To investigate whether K_{ATP} channels are present in human follicles, we used organ culture and molecular biological approaches. Scalp follicles were incubated in media ± minoxidil, tolbutamide (potassium channel closer) or a combination and measured for 9 days. Gene expression...
of the 2 types of $K_{ATP}$ channel components, regulatory sulfonyleurea receptors (SUR) and pore-forming units (Kir) were investigated by RT-PCR.

Follicle growth rate was inhibited by tolbutamide (1mM) and high concentrations of minoxidil (1mM); combined treatment increased inhibition. Only tolbutamide decreased anagen follicle number. Scalp follicles expressed genes for SUR1, SUR2B, Kir6.1 and Kir6.2.

This first report of human follicle inhibition by tolbutamide indicates a biological response to potassium channel closers in vitro; anagen shortening is the opposite of PCO’s anagen prolongation in vivo. Minoxidil’s reported contradictory effects in vitro may be due to culture conditions opening channels. Two forms of $K_{ATP}$ channels are expressed in human hair follicles; only SUR2B channels respond to minoxidil. These results suggest: minoxidil acts directly on human follicles via $K_{ATP}$ channels; novel drugs acting via SUR1 channels alone, or with drugs acting via SUR2B channels, could stimulate greater hair growth; tolbutamide may have applications in suppressing hair growth.

P-152  
Dermal Trichophytic Granuloma  
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The fungi causing tinea capitis, rarely in the immunocompromized state invade the dermis causing granulomatous reaction. Two cases of dermal trichophytic granuloma that invaded the deep dermis and extended to the regional lymph nodes are presented. The cases followed the prolonged faulty use of systemic and local steroids. The presentation, diagnosis and management of the case was real dilemma. Complete recovery followed the stoppage of use of steroids and initiation of systemic antifungal treatment.

P-153  
A Tool From the Nature to Treat Diffuse Alopecia  
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A natural product made from organically cultivated pure essential oils helps to activate dormant hair follicles and revive hair growth. It is a gel containing natural plant sources in an essential oil blend. A study was conducted in our center to evaluate the efficacy of this product in the management of diffuse alopecia.

P-154  
Treatment of Hirsutism with Topical Eflornithine: Our Experience  
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Introduction: Hirsutism is caused by an excess production or action of androgens, usually by the ovaries or adrenal glands. The two most common causes of hirsutism are polycystic ovary syndrome (PCOS) and idiopathic hirsutism. Eflornithine is a specific, irreversible inhibitor of the enzyme ornithine decarboxylase which is thought to slow hair growth by inhibiting this enzyme in hair follicles.

Objectives: the purpose of our study is to evaluate the effect of topical eflornithine for the treatment of hirsutism.

Approach: our treatment regimen was application of a thin film of eflornithine (as 11.5% cream) to the affected areas of the face twice daily for 12 weeks in a group of 20 patients and placebo cream in the other one, without any other topical, systemic or mechanical treatment. We also used a self-assessment questionnaire to assess the effects of treatment on patient well-being.
Results: after 12 weeks' treatment approximately 48% of women treated with eflornithine reported marked improvement in facial hirsutism, compared with 6% of women treated with a placebo cream. Besides, self-assessment questionnaire showed that eflornithine reduced the mean level of overall discomfort and bother. We had just one case of burning and erythema.

Conclusion: Improvement in facial hirsutism can be seen within 4 to 8 weeks after starting topical treatment with eflornithine. Hirsutism may return to pretreatment levels about 8 weeks after discontinuing the medication. Eflornithine is not a depilatory agent; rather, it retards hair growth and could be used in addition to the other hirsutism treatments.

**P-155**

**Distinct Expression of Estrogen Receptor Alpha and Beta in Different Regions of the Human Hair Follicle of Male Versus Female**

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Objectives: In this study, we investigated estrogen receptors (ER) expression and gene regulation of 1) male versus female, 2) frontotemporal versus occipital region and 3) human scalp skin versus hair to further explore gender, scalp location and hair follicle region dependence of estrogen (E2) effects on human hair follicles.

Methods: Skin samples obtained from patients undergoing plastic surgery were collected and either fresh frozen and used directly for RNA isolation or embedded and used for cryosections. Using Laser Microdissection and Pressure Catapulting (LMPC), we microdissected three different regions of the hair follicle (dermal papilla, hair matrix and bulge region) from cryosections of hair follicles from males and females, isolated total RNA from the samples, and then analyzed mRNA expression levels of ER by TaqMan. We also examined an additional 70 genes which are potential HF growth modulators. Immunohistochemistry was performed for ER-alpha and ER-beta protein expression.

Results: Contrary to previously published data, we found stronger ER-alpha mRNA expression than ER-beta mRNA expression in scalp skin samples. We also found that ER-beta mRNA expression in the hair follicles varied in the microdissected regions (dermal papilla, hair matrix, bulge region) and the distribution pattern was gender and scalp location dependent. ER-beta immunoreactivity differed between hair follicle regions, while ER-alpha protein was overall weakly expressed in skin and hair. Of 70 genes tested, several genes were expressed in a sex-dependent manner.

Recognition of the E2-dependent gene regulation will be crucial for the development of more effective gender-tailored management strategies for female versus male pattern balding.

**P-156**

**The Effects of POMC Peptides on the Immune System of the Hair Follicle**

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Background: Hair follicle is a widely available and instructive immune privilege(IP) mini organ in human body that it can be used for a model of studying the maintenance, collapse and restoration of IP. And there are various regulation factors acting on the generation, maintenance, and collapse of hair follicle IP. It is well known that neuropeptides originated from Proopiomelanocortin(POMC) are created in many organs including skin and display various immune regulation effects.

Objectives: To investigate the phenotypic effect of POMC peptides on the hair follicle IP.

Approach: first, we used a potent catagen inducer-interferon-g to make ectopic MHC class I expression hair follicle model in cultured human hair follicles, and then, we examined the effects of POMC peptides on the regulation of ectopic MHC class I expression in cultured human hair follicles using reverse transcriptase-polymerase chain reaction(RT-PCR) and immunohistochemical stain technique.

Results: we showed ectopic MHC class I expression in human anagen hair follicle can be normalized by treatment with adrenocorticotropic hormone(ACTH).

Conclusion: POMC peptides-ACTH are promising candidates for immune privilege restoration.

**P-157**

**A Comparative Study on the Efficacy of Alexandrite and Diode Lasers For Hair Removal in Iranian Women**

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Background: Hirsuties is a common problem in the middle east,especially Iran. As many epilatory methods lead to transient and unwanted effects like pseudofolliculitis,
advances in laser technology are admired by the patients demanding a relatively permanent method of hair removal. We aim to compare the clinical efficacy and side effects of hair reduction of Diode (Nidek) and Alexandrite (Gentlelase) laser systems.

**Methods:** 105 women with Fitzpatrick skin types 2-4 and terminal facial hair were recruited into 2 separate groups who underwent 5 monthly laser-assisted hair removal sessions with either Gentlelase Alexandrite laser (755 nm, 2-msec pulse, 18 mm spot, range of fluences used, 14-20 j/cm²) or a Diode Nidek laser (785 nm, 25 msec, 5 mm spot, range of fluences used 58-61.1 j/cm²). Follow-up hair density counts and subjective satisfaction results of each area were obtained at each of the 5 treatment visits.

**Results:** After about 3 laser treatments, hair counts were reduced to the degree that no shaving or plucking process were required by most of the patients. At the end of 6 months follow up period a mean degree of, 60% improvement in hirsuties score was occurred in nearly equal amounts in both treatment groups. 2 months after the 5th and last treatment, mean degree of clinical hair reduction was observed to be about 60% with no significant differences between the laser systems and fluences used; the patient’s satisfaction score was high especially in Alexandrite group of treated patients.

**Conclusion:** No significant differences were observed between alexandrite and a diode laser for hair removal with fortunately minimal adverse sequelae (1 keloid scarring which improved with intralesional triamcinolone injections). Long-term acceptable hair reduction can be obtained in most patients after a series of laser treatments.

### P-158

**Relationship Between IGF-I and Other Various Factors to Control Hair Follicle Growth**

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**Objectives:** Insulin-like growth factor – I (IGF-I) share high degree of structural and functional homology with insulin and is a potent mitogen supporting cell growth and survival in many kinds of the tissues and cells. It also plays a role in some differentiation and anti-apoptotic function. IGF-I stimulate hair follicle growth, maintain anagen stage and postpone catagen stage. But exact mechanism of effect of IGF-I on hair follicle growth is not yet clearly proved. We investigated the relationship between IGF-I and other various factors (ex. apoptosis related molecules, pro-inflammatory cytokines, other growth factors, etc.) to control hair follicle growth.

**Approach:** We performed hair follicle organ culture with two experimental groups. One is IGF-I treated group and the other is control group. We also performed RT-PCR at 2, 4, 6, 8 day of organ culture.

**Results:** We observed that IGF-I increased PDGF-A, PDGF-B and expression ratio of Bcl-2/Bax.

**Conclusion:** These results show that effect IGF-I on the hair growth is related with anti-apoptotic effect of IGF-I and up-regulation of PDGF-A and PDGF-B.

### P-159

**Topographical Expression of Msx1 and Msx2 in Sheep Skin**

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The size, density and growth duration of hair and wool follicles vary between body sites. In sheep, control of this topographic variation could lead to improved wool value and animal welfare. Two homeodomain-containing transcription factors, Msx1 and Msx2, have previously been linked to regional specification within the skin and also act as regulators of differentiation within the hair follicle matrix. We have characterised the expression patterns of Msx1 and -2 by real-time PCR in multiple body sites of adult sheep and in foetuses at the time of follicle primordia formation. In adult skin, both anterio-posterior (P<0.01) and dorso-ventral (P<0.001) gradients were observed in Msx1 expression across the trunk. However, mRNA of both Msx1 (P<0.001) and Msx2 (P<0.001) were four-fold lower on the face than the belly. Higher concentrations of Msx1 and -2 transcripts were present in adults than in foetuses, possibly reflecting additional roles in regulating the differentiation and growth of the hair shaft. In foetal skin of 70 days gestation, the expression of both Msx1 (P<0.01) and Msx2 (P<0.01) was greater on the belly than the midside. Msx1 expression was five-fold (P<0.001) and Msx2 two-fold (P<0.001) greater on the face than the belly. Thus, Msx expression was greatest in regions with more advanced follicle development, but lower eventual follicle density and fibre growth. These correlative data are consistent with the involvement of Msx1 and -2 in the development of skin regions, follicle growth, and anterio-posterior and dorso-ventral gradients of fibre growth characteristics in sheep.
P-160
Hair Follicle Melanocytes Do Not Constitutively Express MHC Ia or II Antigens

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The anagen hair bulb is one of the few sites of immune privilege (IP) in mammalian tissues. A collapse in hair follicle IP, with increased expression of major histocompatibility complex (MHC) class Ia and MHC class II, is thought to be the mechanism behind alopecia areata (AA) resulting in patches or widespread areas of alopecia. In contrast, the autoimmune attack seen in vitiligo results in depigmented skin. Despite a common autoimmune target, the melanocyte, these two diseases are infrequently co-expressed and rarely co-localised. As such, we hypothesized that there must be fundamental antigenic differences between melanocytes of the skin and those of the hair follicle. In order to examine ex-vivo expression of MHC antigens on melanocytes of the hair follicle and epidermis, we performed double immunofluorescent staining on scalp cryosections from normal subjects with either w6/32 or CR3/43 and NKI/beteb recognizing MHC-Ia or MHC-II expression and melanocytes respectively.

We found that anagen hair follicle melanocytes residing above the apex of the dermal papilla do not express MHC-Ia or MHC-II. Epidermal melanocytes however, do express MHC-II. Due to the strong staining with w6/32 by epidermal keratinocytes, NKI/beteb-labeled melanocytes could not be accurately identified through double-staining and their expression of MHC-Ia could not be conclusively determined.

Our finding of a contrasting MHC expression pattern between these anatomically distinct melanocyte populations may further our understanding why hair follicle melanocytes are preferentially attacked in AA whereas epidermal melanocytes are the target in vitiligo.


P-161
Adenosine Stimulates Fibroblast Growth Factor-7 Gene Expression in Dermal Papilla Cells and Its Contribution to Hair Elongation

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Adenosine is known to play various physiological functions through its receptors’ mediated signal-transduction pathway, in various cell types including dermal papilla cells (DPCs) in hair follicle (1). We performed DNA microarray analyses of DPCs with or without adenosine, and found that adenosine stimulates fibroblast growth factor-factor-7 (FGF-7) gene expression levels by greater than 2-fold. Elevations of the extracellular FGF-7 protein levels were also observed. These upregulations of FGF-7 both at mRNA and protein levels were inhibited by A2b adenosine receptor-specific antagonist, alloxazine, but not by antagonists for other subtypes. In addition, the intracellular cAMP levels were raised by adenosine in a dose-dependent manner. Moreover, an increase of intracellular cAMP augmented the FGF-7 upregulation. Taken together, these results show that adenosine treatment of DPCs upregulates FGF-7 expression via the A2b adenosine receptor and that cAMP acts as one of the second messengers in this pathway. Furthermore, treatment with FGF-7 at concentrations of 10 ng/ml or greater significantly stimulated hair fiber elongation in human scalp hair follicle organ cultures. These data imply that adenosine stimulates hair growth through FGF-7 upregulation in DPCs.


P-162
Repeated Hair Depilation Does Not Alter the Hair Growth Cycles of C3H Mice

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The mouse is commonly used to investigate the hair cycle because of its predictable hair cycle and synchronized hair growth. The murine hair growth (anagen) can also be stimulated by depilation, a procedure that results in all telogen hair follicles entering the anagen stage at the same time. In the current study, we examined the effects of repeated depilation on the hair cycle in C3H mice. Male C3H mice, 46-60 days old in telogen phase, were depilated with a commercially available hair removal wax. Once
the hair follicles in the depilated area were in anagen VI (defined by the presence of grossly evident new hair shafts), a group of these mice were depilated a second time. Results were compared to the mice that had been depilated only once. Examination of the twice-depilated mice defined injury to the hair shafts and hair follicles; however, the injury was repaired over a 7 day period and the hair follicles remained in the identical stage as the single depilation mice. In spite of the hair follicle injury and regeneration, the twice-depilated mice entered the catagen phase at the same time as the mice that had only been depilated once. These findings demonstrate that the depilated anagen hair follicles can repair the injury caused by hair plucking and regenerate the follicles without immediately entering catagen or telogen. Depilation of the anagen hair does not alter the programmed hair cycle initiated by the first depilation.

P-163
Identification of Beta Catenin-Regulated Genes in Human Hair Outer Root Sheath Cells Cultured in Vitro
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The Wnt signaling induces various cellular responses, including cell proliferation, fate determination and terminal differentiation, through the stabilization and accumulation of b-catenin. Although b-catenin has been recognized to be a key molecule in the hair follicle formation and anagen induction, the downstream effectors of b-catenin have not been clearly defined yet. To identify the b-catenin-regulated genes, we made a recombinant adenovirus harboring the expression cassette for b-catenin. After adenoviral transduction into the human hair outer root sheath (ORS) cells cultured in vitro, the increase of cellular b-catenin was confirmed by Western blot analysis. Then, we isolated total RNA and performed cDNA microarray. We chose the genes that showed at least two-fold induction by b-catenin. The expression of selected genes was verified using RT-PCR. As a result, we identified several b-catenin-regulated genes in ORS cells, including keratin 15, 16 and 17. In addition, the expression of some histone deacetylases, such as HADC1, 2, and 3, were significantly affected by the overexpression of b-catenin. The roles of b-catenin-regulated genes in ORS cells will be investigated further. Our results provide important clues, on which to base further investigations of hair growth modulation by b-catenin.

P-164
Expression of Ephrin-A and Epha Family in Hair Follicles and Effects of Ephrin-A-Epha Oversignaling on Hair Formation and Differentiation
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Objectives: We have already reported that ephrin-A3 was markedly down-regulated in dermal papilla cells from androgenetic alopecia patients. Although ephrins are known to regulate a variety of developmental processes, little is known of their role in hair development. So we investigated the expression of ephrin-As and whose receptors (EphAs) in hair follicles throughout the hair cycle. We also tried to clarify the role of ephrin-As – EphAs signaling on hair follicle formation and differentiation.

Approach: We studied the expression of ephrin-As and EphAs in C3H/He mice from the day of birth to the second hair cycle with qPCR and immunofluorescence methods. Next, ephrin-A3 was subcutaneously injected into neonatal mice and the effects of ephrin-A3-EphAs over-signaling on hair formation and differentiation were studied morphologically.

Results: ephrin-A1, A3, A5, and EphA4 were expressed synchronously with the hair cycle and were localized in outer root sheath at anagen phase and secondary hair germ at telogen-anagen transition phase. Subcutaneous injection of ephrin-A3 into neonatal mice markedly accelerated differentiation processes of hair follicles and increased the hair follicle number; this was not a transient phenomenon but was also conserved in the second hair cycle.

Conclusion: All findings of these studies revealed the characteristic spatiotemporal expression of ephrins and Ephs in hair follicles through the hair cycle, and also indicated that ephrin-A3 not only accelerates the hair follicle development, but also increases the density of hair follicles. Ephrin-A3 is the first molecule presumed to be involved in hair follicle differentiation and formation.
P-165
Whole-Mount Detection of Apoptosis and Proliferation in Human Hair Follicles by 2-Photon Laser Microscopy
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In recent studies, we have correlated cutaneous apoptosis and proliferation during hair follicle (HF) morphogenesis and cycling demonstrating the crucial role in sculpturing the HF structure in mice (Magerl et al., J Invest. Dermatol. 2001,116:947). Here we introduce a pioneering technique to overcome the limits of traditional two dimensional staining methods by using whole mount imaging of dissected human hair follicles visualized by deep penetrating two-photon laser microscopy.

With the novel whole-mount in situ technique, TUNEL and Ki67-immunoreactivity were combined to investigate the spatiotemporal patterns of apoptosis and proliferation within micro-dissected human hair follicles. In addition, we were capable to detect adjacent blood vessels as well as collagen and elastin fibres. These structures were co-visualized by second harmonic generation and auto fluorescence imaging. With this experimental setup, key stages of the human hair follicle cycle were analysed and three-dimensional maps were generated by software rendering.

Beside massive up-regulation of Ki67-positive keratinocytes seen in the proximal hair matrix of anagen HF, clusters of Ki67-immunoreactive cells were seen in the distal portion of the HF in close vicinity to the putative stem cell region. Consistent to previously described expression patterns in mice, bundles of strong TUNEL positive cells were seen in the trailing epithelial strand in human catagen HF.

Three dimensional follicular imaging has highlighted an unexpected degree of dynamics and turnover of growth and regression processes within the human HF as well as remodelling of connective tissue underlining the vital function in shaping HF topology.

P-166
The Immune Privilege of Human Hair Follicles Is Selective and May Not Include Resistance to Complement Activation
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The immunology of hair follicles and their role in the skin immune system has significant impact both biologically and clinically. The hair bulb part during anagen is believed to retain immune privilege (IP) characterized by very low expression level of MHC I, suppression of MHC II-dependent antigen presentation, and production of immunosuppressive agents. We generated a list of 19 immunoregulatory genes, and determined their differential expression in human hair follicles (HF) as compared to normal skin epithelium. We performed microdissection of HF from scalp biopsies of normal individuals, and further dissected the tissues into bulb (B) and shaft (S) parts. RNA synthesis was then conducted, followed by reverse transcription and, finally, real-time RT-PCR was conducted. Consistent with previous publications, the immunosuppressive gene a-MSH was significantly upregulated in both bulb and shaft (2.06 fold, and 4.26 fold respectively). Consistent with a lack of lymphocytes and antigen presentation in HF, CD80 (B: 5.30 fold; S: 3.99 fold) and CD86 (B: 4.09 fold; S: 1.60 fold) were downregulated. The apoptotic genes Fas (B: 2.46 fold) and Fasl (B: 78.71 fold; S: 7.79 fold) were both downregulated significantly in our analysis. Also, we found that complement suppressive factors, such as CD 55 (B: 5.48 fold; S: 3.52 fold) and CD59 (B: 2.29 fold) were downregulated. These results reveal some putative candidates that participate in the IP of hair follicles, but HF IP is selective and, notably, the ability to blockade complement activation may actually be reduced as compared to normal skin epithelium.

P-167
Uneven Proteoglycan Distribution Along the Human Hair Follicle
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Background: Proteoglycans are known to play a key role in many cellular signaling pathways involved in hair follicle biology. Although proteoglycans were well described in rodents, they were incompletely studied in adult human hair follicle.

Objectives: To review and complete description of proteoglycans and glycosaminoglycans in human anagen hair follicle and for selected one during the hair cycle.

Approach: We used immunohistochemistry and immunohistofluorescence to revisit the expression pattern of glycosaminoglycan chains and core-proteins in human hair follicle. The studied epitopes included CD44v3, syndecan, perlecan, versican, aggrecan, heparan sulfates, chondroitin sulfates and keratan sulfate.

Results: Our results confirmed and extended the notion that both connective tissue sheath and dermal papilla contained high amounts of proteoglycans such as perlecan, versican, heparan sulfates or chondroitin sulfates. We also observed a varying distribution of these components along the hair follicle. Especially, we noted a proteoglycan impoverishment
at the very bottom of the bulb. During catagen, 4C3 and PG4 chondroitin sulfate epitopes disappeared in dermal papilla and connective tissue sheath respectively, confirming the arrest of extracellular matrix synthesis during this regression phase.

Conclusion: Uneven expression along the anagen follicle and remodeling of some epitopes during hair cycle suggest that proteoglycans are involved in nutrient diffusion, cell proliferation and differentiation, and hair protection.

P-168
Activin A and Follistatin Influence Hair Follicle Development in Mice
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Members of the TGFβ superfamily are known modulators of hair follicle development. In particular activins and follistatin are highly-expressed in the skin at critical phases of hair follicle formation. To ascertain if these molecules are causally related to hair follicle induction, we determined the effects of exogenous activin A and follistatin on the development of tylotrich and non-tylotrich hair follicles in embryonic mouse skin cultures. Changes in gene expression associated with treatment with activin A were also quantified by quantitative PCR of candidate genes involved in TGFβ superfamily signalling in a subset of the animals.

Skin explants were collected from 13.5 and 15.5 days post coitus (dpc) mouse embryos and cultured for 3 days in the presence or absence of recombinant human activin A (rhActA) or follistatin (rhFS300). While there was no significant effect of rhActA on follicle density in 13.5dpc skin, there was a 10-17% decrease in follicle density of 15.5dpc skin cultured in rhActA compared to controls (P<0.05). However, there were no changes in the gene expression in rhActA-treated explants, when normalized against three housekeeping genes. While rhFS300 supplementation had no effect on follicle density, rhFS300 did reduce the extent of follicle downgrowth and delayed follicle maturation in the 13.5 dpc-derived explants.

These results suggest activin A is involved in follicle initiation of non-tylotrich hair follicles, most likely through post-translational modifications of components of the TGFβ signalling pathways, rather than via gene expression. Follistatin appears to influence follicle maturation and downgrowth through mechanisms as yet unknown.

P-169
Expression of Pitx2 in Human Hair Outer Root Sheath Cells
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Pitx2, a bicoid-type homeodomain transcription factor, has been shown to play a central role in differentiation and development. In the human, four isoforms of Pitx transcripts has been isolated, which are named as Pitx2a, b, c and d. Pitx2a, Pitx2b, and Pitx2c are structurally related, differing only in their amino terminal region. Pitx2d shows somewhat different structure, and acts to repress the activity of the other Pitx2 isoforms. Among those, Pitx2c isoform was predominantly expressed in normal human epidermal keratinocyte cultured in vitro. In this study, we investigated the expression of Pitx2 in hair follicle using immunohistochemistry analysis. The expression of Pitx2 is detected predominantly in outer root sheath (ORS) of lower half of hair follicle. To investigate the role of Pitx2 in ORS cells, we made a recombinant adenovirus harboring the expression cassette for GFP-Pitx2c fusion protein. Primary cultured ORS cells were transduced with recombinant adenovirus and then Pitx2 expression was detected in the nucleus. To identify the Pitx2-regulated genes, we performed RT-PCR and Western blot analysis for several intermediate filament proteins and cell cycle-related proteins. Interestingly, overexpression of Pitx2c in ORS led to the upregulation of keratin 6. In addition, overexpression of Pitx2 resulted in the change of several cell cycle-related protein levels such as p21. These results implicate the potential importance of Pitx2 in the regulation of hair growth.

P-170
Substance P Receptor Expression in Human Hair Follicles
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Background: Among the constituents of skin, hair follicle is an organ which has the highest density of nerve fibers distribution. It has been reported that neuropeptides which are secreted by nerve fibers have important roles in the hair growth and hair cycle change. Substance P (SP), as a neuropeptide distributed over the wide human skin that displays its effect through combining with special receptor. In the previous study conducted by us, we showed
that SP had a positive effect on the hair growth and especially prolonged the anagen phase in culture of human hair follicles.

Objectives: To investigate the expression of substance P receptor (SPR) in human hair follicles, hair follicle cells and its expression change when treated with SP and SPR antagonist – spantide I.

Approach: Human hair follicles and hair follicle cells were cultured. The expression of SPR was examined by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical stain. The expression change of SPR in human hair follicles was detected by RT-PCR and Western-blotting analysis.

Results: SPR expressed in human hair follicles and hair follicle cells, SP upregulated the expression of NK-1R, SPR antagonist -spantide I can reduce the expression of NK-1R.

Conclusion: In culture of human hair follicles, SP, through combining with special receptor-SPR displays the effects of prolonging the duration of anagen phase.

P-172
Endostatin Overexpression in the Skin Interferes With Hair Follicle Development
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Objectives: To study how overexpression of endostatin, the C-terminal domain of collagen XVIII, affects hair follicle morphogenesis. Collagen XVIII is a basement membrane (BM) proteoglycan, suggested to have a role in maintaining the structural integrity of BMs. Endostatin is also known to affect integrin- and VEGF-mediated signaling.

Approach: Mice overexpressing endostatin in the skin under the keratin-14 promoter (ES-tg) and control FVB/N mice were used in the experiment. Skin samples were collected from the neck region of control and ES-tg male mice (n=5 per group) at different timepoints after birth (1-32 days). HE- and immunofluorescence stainings were performed.

Results: At postnatal day 1 the hair follicles in both mouse lines were positively stained for collagen XVIII. However, in the ES-tg mice reduced intensity of the staining was detected with an antibody specific for the two longest variants of collagen XVIII. These include a variant with a cysteine-rich domain homologous to the frizzled-proteins which potentially binds to Wnt-proteins. At day 25 the hair follicles of control mice reached deep to the subcutis to the close vicinity of the panniculus carnosus. However, in the ES-tg mice the hair follicles were not found as deep in the subcutis, and the follicles also appeared thinner and fewer in number. The analyses from the other timepoints are currently ongoing.

Conclusion: Based on our results, we suggest that excess of endostatin leads to abnormal hair follicle development.
P-173
Morphological and Immunohistochemical Examinations of Human Hair Follicles: Special Reference to the Bulge Area
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We have focused the characterizations of the bulge areas of human hair follicles where follicular stem cells may be present. Serial transverse sections of human scalp skin were observed. In the terminal hair follicles of the human scalp, we found apoptotic pocket-like structures in the outer root sheath of the bulge area at anagen, but not telogen phase. The size of these apoptotic pockets was variable, ranging from pin hole-like spaces to larger structures. Moreover, the distributions of Merkel cells, Langerhans cells and melanocytes within human hair follicles were immunohistochemically examined. Merkel cells were stained with CK20, while Langerhans cells were stained with CD1a. Melanocytes were also decorated with NKV beteb.

We immunohistochemically confirmed the localization of Merkel cells, Langerhans cells and melanocytes within the bulge areas in normal human hair follicles. Using a double staining technique, it was clearly demonstrated that a small proportion of Merkel cells were closely contacted with Langerhans cells below the sebaceous gland level, presumably indicating the bulge area. Merkel cells and Langerhans cells connected directly or approached each dendrite within the basal layer of the outer root sheath. We demonstrated a close anatomical relationship between Merkel cells and Langerhans cells and pocket-like structures within the bulge area of human hair follicles.

P-174
Transforming Growth Factor-B Receptor II is Preferentially Expressed in the Companion Layer of the Human Anagen Hair Follicle
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Transforming growth factor beta (TGF-b) is a multifunctional growth factor with multiple roles in skin including hair follicle development and cycling, where it regulates cell proliferation, differentiation and apoptosis, as well as in wound healing. While TGF-b RI and TGF-b RII expression helps define early human hair follicle morphogenesis, expression in the adult human hair follicle remains to be established. The aim of this study was to assess TGF-b receptor expression in human scalp anagen hair follicles. Immunohistochemical and double immunofluorescence analysis of TGF-b RI and RII was conducted on frozen sections of haired human scalp obtained from 10 healthy individuals.

TGF-b RI expression was detected in the outer root sheath of anagen hair follicles while TGF-b RII was expressed almost exclusively in the companion layer of inner root sheath and less so in pre-medulla keratinocytes. Both receptors were co-localized in the companion layer of the proximal and mid follicle. The well-described role of TGF-b in keratinocyte apoptosis during catagen is likely to involve anagen-specific hair follicle components including the companion layer as this layer provides the slippage plane supporting the inner root sheath and hair shaft as they ascend to the skin surface. Results of this study suggest that the co-localization of TGF-b RI / RII complexes at the companion layer would facilitate TGF-b signalling at this site to regulate apoptosis of the companion layer keratinocytes, facilitating shrinkage/contraction of this cell layer during hair follicle regression / catagen.

P-175
Leptin Is a Paracrine Regulator of Hair Cycle
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Leptin, one of adipokines secreted from adipocytes, is known to affects energy balance by interacting with hypothalamus. Recent studies have shown that non-adipose tissues also produce leptin. Leptin acts on target tissues via specific receptors (Ob-Rs), and activates STAT3. We have previously reported that the activation of STAT3 is indispensable for hair cycle progression. In this study, we examined whether leptin is physiologically involved in hair cycle progression using well-established diabetic model mice. Back skin of 5 wk old C57/BL6 mice showed late anagen phase, while the hair follicles of Ob-Rb deficient Db/Db mice were still in the first telogen phase, indicating the inhibition of the second hair cycle progression. Hair plucking of back skin induced the anagen phase in 7 wk old C57/BL6 mice, but Ob/Ob mice showed no hair growth 3 weeks after plucking. In Ob/Ob mice, the angen phase was induced 1week after plucking concomitant with local leptin injection. Moreover, anagen was induced in the injected site 3 weeks after local leptin injection in 7 wk old C57/BL6 mice. The production of leptin by cultured human dermal papilla cells were confirmed by RT-PCR and ELISA. Phosphorylations of ERK, JAK2, and STAT3 in cultured human keratinocytes were examined by western blot using specific antibodies for phosphorylation sites. All
of the molecules were phosphorylated by leptin treatment. These results indicate that leptin is physiologically involved in hair cycle progression, and suggest that the anagen phase is induced by leptin from dermal papilla cells in a paracrine manner.

P-176
The Notch Signaling Pathway Is Involved in Wool Follicle Development

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Wool follicle development is initiated during fetal life through signals exchanged between the mesenchyme and epithelium. Follicle primordia are initially visible as condensations of dermal prepapilla cells associated with epithelial thickenings. Notch genes are expressed in fetal skin and the signal pathway is involved in cell fate specification.

This study investigates the role of Notch in specifying the papilla cell population, during initiation in the sheep, using in situ hybridization (ISH) and cell culture.

Ovine skin samples were collected from fine-wooled Merino and strong-wooled Tukidale fetuses (day 56 and 70 of gestation). ISH studies were conducted using a DIG-labelled riboprobe generated from a murine Delta-1 cDNA sequence. Counts of specifically labeled cells were analysed. Dermal papillae (DP) harvested from adult Merino whisker follicles were cultured in vitro. Cultures were treated with a g-secretase inhibitor (DAPT) that blocked Notch signaling to determine if it affected cell aggregation.

ISH results revealed the presence of Delta-1 transcripts prior to and during follicle initiation. Significant differences in the numbers of labeled cells were found during initiation. At this time, the percentage of labeled cells was 41.54 ± 1.60 in the Merino, compared to 46.22 ± 2.22 in the Tukidale. In vitro studies showed that DAPT inhibited DP cell aggregation, presumably through Notch signal blockade.

We conclude that Notch pathway genes expressed in mesenchymal cells are involved in specifying prepapilla cell fate at follicle initiation.

P-177
Keratin Gene Expression Varies Between Sheep Breed, Primary and Secondary Wool Follicles and with Follicle Growth Status

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Mammalian fibres are predominantly comprised of keratins and their associated proteins, hence the expression and translation of the corresponding genes will underlie many fibre characteristics. Recent rapid progress in the understanding of the genomic structure of keratin genes and their expression in the human hair follicle have provided a basis to study the mechanisms of hair and wool fibre formation. Skin samples were collected from New Zealand Wiltshire, Romney and Merino sheep, and from wool follicles in synchronised stages across the hair cycle. The expression patterns of five intermediate filament and six keratin-associated protein (KAP) genes were investigated by qPCR, Northern blot and in situ hybridisation. Significant differences in expression between sheep breeds, follicle growth stages, and between primary and secondary follicles were found. While expression of the follicle-specific genes fell rapidly during catagen, only some keratin intermediate filaments (KRT27, KRT31 and KRT85) and KAPs (KRTAP6.1 and KRTAP8.1) appear to be involved in brush-end follicle formation. Skin samples were collected from New Zealand Wiltshire, Romney and Merino sheep, and from wool follicles in synchronised stages across the hair cycle. The expression patterns of five intermediate filament and six keratin-associated protein (KAP) genes were investigated by qPCR, Northern blot and in situ hybridisation. Significant differences in expression between sheep breeds, follicle growth stages, and between primary and secondary follicles were found. While expression of the follicle-specific genes fell rapidly during catagen, only some keratin intermediate filaments (KRT27, KRT31 and KRT85) and KAPs (KRTAP6.1 and KRTAP8.1) appear to be involved in brush-end follicle formation. Localisation patterns for KRT31, KRT35, KRT38, KRT85, KRTAP4.3 and KRTAP6.1 differed between primary and secondary follicles, and qPCR showed higher levels of expression of these genes (apart from KRTAP6.1) in body sites with lower S:P ratios and lower follicle densities. Further studies are underway to link gene expression with wool protein composition and, in turn, to the control of fibre formation. An improved understanding of the regulation of all the major wool and hair keratins and KAPs will contribute to the development of novel fibres, new cosmetics and keratin-based industrial applications.

P-178
Gene Expression Pattern Similarity Between Hair Follicles and Basal Cell Carcinomas

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Hair follicles and BCCs can be regarded as ordered and disordered skin appendages respectively, and may utilize similar molecular mechanisms of growth. We wanted to examine the similarities and differences in gene expression patterns between BCCs and hair follicles to define common growth mechanisms and patterns that distinguish an
ordered skin appendage from a disordered skin growth. Nodular cystic BCCs (n=8) and non-follicular skin epithelium (n=8) were obtained from previously untreated patients undergoing Mohs surgical resection. Scalp hair follicle epithelial root sheath was micro dissected from between the sebaceous gland duct and the lower one third of the hair follicle (n=7). Microarray analysis was performed using 21K sequence verified cDNA arrays and selected genes were validated using quantitative PCR. Two differentially expressed genes sets were identified by T-test and 1.5 fold changes filtering from BCC and hair follicle epithelium verses skin epithelium respectively. Based on these two lists, 1429 similarly expressed genes in hair follicles and BCCs were obtained and applied to multiple signaling pathways analysis. Analysis indicated that Notch signaling, apoptosis, hedgehog, WNT, and TGF beta pathways were involved in regulating formation of hair follicles and BCCs. In particular, Notch signaling, including Notch 1, Notch 2, DTX2 and DNER, showed selective differential activation in BCCs and hair follicles. Our data provides compelling evidence that “tumorgenic” growth signaling pathways are commonly expressed in both hair follicle epithelial progenitor regions and BCCs. However, in hair follicle 3 times the number of genes is differentially expressed as compared to BCCs.

**P-179**

**Characterization of Wnt Ligands Important For Hair Follicle Development**

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Multiple Wnt ligands are expressed in embryonic skin at the time of hair follicle placode initiation, and several of these are upregulated in placodes and in specific subsets of hair follicle cells at subsequent developmental stages. Analysis of transgenic mice expressing Wnt/β-catenin reporter genes has indicated that Wnt/β-catenin signaling is active at several stages of hair follicle development. Forced expression of the potent secreted Wnt/β-catenin pathway antagonist Dickkopf1 (Dkk1) prevents initiation of hair follicle placode development, demonstrating that Wnt/β-catenin signaling plays a critical role in this process. However, the specific Wnt ligands required for hair follicle development have not been identified. To begin to address this question we are analyzing skin and hair follicle development in mice carrying loss of function mutations in Wnt genes that are expressed in the embryonic skin and hair follicles. Analysis of mice lacking epithelial Wnt3, that is broadly expressed in the embryonic epidermis, revealed defects in limb development, but normal hair growth. Similarly, hair follicle development was grossly normal in mice lacking Wnt10b, the Wnt showing the earliest specific localization to developing placodes. As expression of Wnt10b overlaps with that of a related Wnt, Wnt10a, we have now generated mice lacking Wnt10a. Preliminary analysis suggests that mice lacking Wnt10a die during embryogenesis. We are currently determining the stage of lethality of Wnt10a-null mice, and analyzing the skin and hair follicle phenotypes of Wnt10b-/-; Wnt10a-/- mice.

**P-180**

**No Association Between Serum Ferritin Levels and Hair Loss Activity in Otherwise Healthy Women**

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**Background:** Hair loss is common in women. Female pattern hair loss (FPHL) and diffuse telogen effluvium (TE) account for most cases. Low iron stores are considered a possible contributing factor, and assessment of serum ferritin levels is recommended as part of routine investigation. However, contradictory data have failed to resolve whether an association exists between hair loss and low ferritin levels.

**Objectives:** To evaluate the relationship between serum ferritin levels and hair loss activity determined by trichogram in otherwise healthy women.

**Approach:** Retrospective case study of 413 consecutive women who presented at the Department of Dermatology, University Hospital of Zurich hair clinic between 2002 and 2005 for assessment of hair loss due to FPHL or TE. All underwent biochemical investigations and trichograms.

**Results:** After exclusion of patients with a history of disease, abnormal laboratory studies (except ferritin), or on drugs known to cause hair loss, 181 women remained. Of these, 112 (62 %) women had a serum ferritin > 30mg/L (lower reference limit of normal for children, men and non-menstruating women), 55 (30 %) between 10 – 30 mg/L, and 14 (8 %) < 10 mg/L (lower reference limit of normal for menstruating women). No correlation was found between serum ferritin levels and pathologic trichograms (telogen rate > 15%).

**Conclusion:** There is no relationship between serum ferritin levels and hair loss activity determined by trichogram. The usefulness of serum ferritin assessment for the purpose of therapeutic iron supplemation in women with FPHL or TE remains debatable.
P-181
Lipid Metabolism By Cutaneous Malassezia Yeasts and Its Implications For Scalp Condition and Dandruff
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Dandruff is a global consumer problem, characterised by flaking and scaling of the scalp, accompanied by itch and irritancy. However, despite much research, the aetiology of the condition remains poorly understood, although there is a strong consensus that yeasts of the genus Malassezia are a major contributory factor. Again, however, there is a paucity of understanding on how this commensal organism adopts a pathogenic phenotype. The objective of these studies was to investigate the metabolism of sebaceous lipids by Malassezia yeasts, a mechanism by which these organisms have previously been reported to generate scalp irritants that mediate dandruff. Assay systems were developed to study the metabolism of triacylglycerols and fatty acids by a representative selection of Malassezia isolates. Malassezia species exhibited lipase activity, hydrolysing triacylglycerols to free fatty acids, while the ability of these yeasts to utilise fatty acids as a source of carbon and energy was also demonstrated. Of particular note, Malassezia species were shown to partially catabolise structurally-unusual (e.g. methyl-branched) fatty acids to chain-shortened species. At face value, these volatile fatty acid (VFA) products may be seen as promoters of scalp itch and irritancy, and thus mediators of dandruff. However, this rather simplistic explanation ignores the many other sources of fatty acids, including VFAs, on scalp skin, as well as information emerging on the complex immunological interaction that exists between Malassezia and its host. Thus, the metabolism of sebaceous lipids by Malassezia yeasts, while of academic interest, is probably not a major aetiologic factor in dandruff.

P-182
The Role of Alimentary Integrators in a Trichologic Context: a Report of Italian Association of Women Dermatologists
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Recent evidence of literature have well established the important role for health of the alimentary integrators contained naturally in food such vitamins, minerals, fibers. The use of these molecules by dermatologists is individual and it doesn’t follow specific guidelines. Usually patients assume these molecules by self prescription following hormonal changes of pregnancy and seasonal climatic changes.

It is demonstrated that the use of elementary integrators containing cyssteine and sulphurated aminoacid as well as of vitamins, antioxidants mineral and free antiradicals like A, B5 vitamins and microelements can be very useful in some stress conditions.

To better understand the role of alimentary integrators in the regimen of patients the Italian Association Women Dermatologists conducted a questionnaire on 285 women investigated the knowledges and the employment of common integrators.

P-183
Iron Deficiency-Induced Hair Loss Due to Incorrect Dietetic Habits in Iran
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Background: Iron deficiency – induced hair loss is very common in Iran even in urban areas,although its incidence in rural regions will be much higher.Statistics show 80% of female Iranians in reproductive age have subclinical iron deficiency although this level would not necessarily cause anemia but would be sufficient to cause hair dryness, fragility and losses.

Discussion: A very common and cultural habit in our citizens is drinking strong tea especially immediately after lunch and dinner,so despite probably enough intake of iron in meat,eggs and similar foods ,this will lead to intestinal malabsorption of Iran. On the other hand in rural areas the red meat availability is limited due to its dear price and cultural or social habits of villagers shift them to eat rice, bread and “beans/vegetable” soup (instead of steak or kebabs made from meat) as main nutrition.

Conclusion: These facts are obtained in our research in detail.

Education and insight to the adverse effects such as hair loss, graying hair and hair shaft fragility which is very important to young Iranian females can possibly reverse such habits effectively and with a low cost.

Key words: Iron deficiency, hair loss, dietary habits, Iranian females
P-184
The Stimulatory Effects of Cepharanthine and Plant Worm on Hair Growth
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Objective: The purpose of this study is to analyze the effect of Cepharnthine (CE), a biscoclaurine alkaloid obtained from the plant, and Plant worm (Cordyceps sinensis) on hair growth stimulation in a C3H/HeN mouse system in vivo. The effect of CE on cell proliferation of dermal papilla cells (DPCs) and outer root sheath cells (ORSCs) in vitro was also analyzed.

Approach: For quantitative evaluation of hair growth in mice, 0.002% CE solution, 0.75% Plant worm solution and vehicle solution (75% alcohol) were applied to the dorsum of the 8 weeks old C3H/HeN mice once daily for three weeks, after clipping the dorsal hair in telogen. In vitro: DPCs and ORSCs were cultured with or without CE for 5 days, and then tested of cell proliferation by alamarBlue™ assay.

Result: Significant effect on hair growth of mice treated with CE and Plant Worm were seen compared with the vehicle treated group (p<0.05, n=10). In addition, 0.1μ/ml CE stimulated cell proliferation of cultured DPCs (p<0.05), but not that of cultured ORSCs.

Conclusion: These results confirmed that CE and Plant Worm were significantly effective in accelerating hair re-growth, otherwise CE might have an effect on DPCs directly.

P-185
Human Scalp Hair Growth Modulation By Dickkopf1
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Objectives: To investigate the effects of Dickkopf 1 (DKK-1) on human scalp hair growth.

Approach: Outer root sheath (ORS) keratinocytes and dermal papilla (DP) cells were cultivated from human scalp hair follicles and treated with recombinant human DKK-1 (rhDKK-1). Hair follicles were also cultured in vitro in the absence or presence of recombinant human DKK-1 (rhDKK-1) in William’s E medium.

Results: rhDKK-1 inhibited the growth of outer root sheath (ORS) keratinocytes but not DP cells. rhDKK-1 also induced apoptotic cell death in ORS cells accompanied by Bax induction. In addition, rhDKK-1 inhibited elongation of hair shafts and caused apoptotic cell death of epithelial cells in cultured hair follicles.

Conclusion: Altogether, our data suggest that DKK-1 may inhibit the human scalp hair growth by triggering apoptosis in follicular keratinocytes.

P-186
Hair Growth Activity of Cosmetic Diaminopyrimidine-Oxide Compound in Vitro & in Murine Anagen Induction
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Many pyrimidine oxide derivatives were known to have the vasoactivity for hypertension and alopecia. But only minoxidil (6-(1-Piperidinyl)-2,4-pyrimidinediamine-3-oxide) was approved from FDA for topical drugs for alopecia.

We investigated the hair growth activity of 2,4-diamino-6-pyrrolidin-pyrimidine-3-oxide (Triaminodil, Proderma, Italy, Cas No. 55921-65-8), which was developed for cosmetic ingredient. We first observed the active effect of triaminodil & minoxidil on NIH3T3 fibroblast in ATP-sensitive potassium channel-dependent fashion and then anagen induction also was evaluated after topical administration on telogen phase murine dorsal area.

In vitro effects of established potassium channel opening were indirectly assessed on NIH3T3 fibroblast in the absence of aminoglycoside antibiotics, phenol red and 5% fetal bovine serum-supplemented medium. 2.5mM tolbutamide inhibited 40% proliferation of NH3T3 fibroblast as reported previously. When minoxidil & triaminodil were treated to the fibroblast with 2.5mM tolbutamide, growth inhibition of NIH3T3 fibroblast was suppressed 51.49% by 100uM minoxidil 58.7% by 100μM triaminodil.

Two topical preparations of single dose (0.5%, w/v) of minoxidil and triaminodil were topically administered on clipped dorsal area of female telogen phase C57bl/6. The grown hair weights of topical minoxidil preparation(52.17±4.99mg, P<0.001) and topical triaminodil (59.27±10.59mg, P<0.001) increased significantly than that of vehicle (14.26±2.51mg). Cosmetic triaminodil compound will be easily used as an effective vasodilator for anti-hair loss cosmetics or quasi-drugs.
P-187
Importance of Reversible and Environmental Factors in Hair Loss in Developing Countries

Sepanj, Giti; Health And Cultural Primary Education Ministry Department, Isfahan, Iran

Riding hairstyles and the arrival of the bald patch are feared by men and especially women around the globe. Not even the efforts of a few superstars to make baldness fashionable have succeeded in releasing men or women from this inherent terror. Bald women are not socially acceptable at all.

Hair may start to disappear from the temples and the crown of the head at any time. For some men this process starts as early as the later teenage, for most it happens in the later 20's and early 30's. Initially it may just be a little thinning that's noticed. Then, the absence of hair allows more of the scalp to become visible.

Some men are not troubled by this process at all. Others, however, suffer great emotional distress associated with a lack of self-confidence and sometimes depression and even suicidal attempts.

Not every hair follicle has the baldness gene which is why some hair falls out whilst other hair doesn’t.

Other causes of hair-loss that are usually reversible include: iron deficiency anaemia especially in middle eastern countries and developing communities around the world, a statistical survey showed 85% of female college students in Isfahan have a ferritin level lower than 50 microg/ml; hypothyroid state may be first present with hair thinning and increased loss; fungal scalp infection (Favus in our country in rural -resident adult patients has been detected more than before; some prescribed medicines (anti malarials which are sometimes prescribed for a mild arthralgia, application of cedar leaf on hair during bathing) and crash diets taken for a slim-body (Barbie) fashion in causes protein malnutrition (i.e. methionine and tyrosine low intake).

All can be prohibited by an educational program in high schools.

Conclusion: An insight to reversible factors such as diet deficiencies and medications can encourage any hair graying control treatment effectively

Key words: Premature graying hair ,Iranian adolescent population, environmental factors

P-188
Premature Graying Hair a Common Problem in Iranian Adolescent Students

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Background: Premature graying of scalp hair is extremly feared by female adolescent people in our country. The hair and its beauty nowadays will be a preoccupation in the youngs. Hair may start to become gray from the temples of the head at any time depending on genetic background. For caucasians, this process may start as early as the early twenties. Most of it though, happens in the later 20's and early 30's. Initially it may not be a cosmetic disaster. As in our culture and even global culture whitening or graying will be a strong sign for old age, it is hated by the youngs and as a statistical survey shows, causes great emotional distress associated with a lack of self-confidence.

Discussion: A few causes of graying hair are usually reversible which increase its incidence dramatically in recent years, including: iron deficiency anemia, folate and Vit B 12 deficiency especially in middle eastern countries and developing communities around the world, (a statistical survey showed 70% of female high school students in our city, Isfahan, have a ferritin level lower than 45 ng/ml); association with hypothyroid state is to be evaluated. Stress sufferers, near national exams for example show rapid and early manifestation in this regard; some prescribed medicines (anti malarials which are sometimes prescribed for a mild arthralgia, application of cedar leaf on hair during bathing) and crash diets taken for a slim-body (Barbie) fashion in causes protein malnutrition (i.e. methionine and tyrosine low intake).

All can be prohibited by an educational program in high schools.

Conclusion: An insight to reversible factors such as diet deficiencies and medications can encourage any hair graying control treatment effectively

Key words: Premature graying hair, Iranian adolescent population, environmental factors

P-189
Oral Supplementation With Taurine in the Treatment of Women With Hair Fragility

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The aim of this study was to evaluate the efficacy of oral supplementation with daily Taurine 150 mg + Catechin 75 mg + Zinc 15 mg (Inneov Trico masse) in the treatment of women with fine hair, hair fragility and decreased hair thickness.

Materials and Methods: The study involved 20 women patients aged from 20 to 68 years (median age 45) who presented to us with fine hair and decreased hair thickness. These women had to take Inneov Trico masse tablets, twice a day, for 6 months.
Assessment was carried out using standardized global photograph of the scalp and measuring, with videodermoscopic images, hair diameter of a target area.

- Videodermoscopic images were acquired by computerized polarized-light videomicroscopy Dermoscope DDS(r) Dermascope 2.0 Software (for measuring hair diameter)
- The target area was a patch of ten hairs. During the first visit the researcher cut hair of the patch. The hair were collected and examine them at month 2, 4 and 6

**Results:** In 11/12 patients that have terminated the 6-month's study, the evaluation of the average of the hair diameter showed increase (from 0.040 to 0.045 μm). 6 other women have interrupted the study to 4 months, 4 patients showed a good increase of the hair diameter (from 0.040 to 0.043 μm).

**Conclusions:** Our study suggests that oral supplementation with Taurine in association with Catechin and Zinc may be helpful in the treatment of woman with hair fragility.

This work was sponsored by a grant from Inneov nutricosmetics Italy

**P-190**

**Iron Deficiency and Alopecia**

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**Objectives:** Iron deficiency (ID) is the world's most common nutritional deficiency and is associated with significant morbidity. Alopecia affects women and men of all ages and can have a significant impact on health and quality of life. We reviewed the literature on the relationship between ID and alopecia.

**Approach:** We used a MEDLINE search from 1965 to 2004 using the subject headings “alopecia” and “iron deficiency.” In addition, we reviewed the relevant references that those articles cited. Studies that used human subjects with clinical endpoints were chosen.

**Results:** Eleven studies matched our criteria. All 11 studies examined females, while 2 examined males. The studies varied widely both in sample sizes and study types. Many suggest that ID may be related to alopecia areata, androgenetic alopecia, telogen effluvium, and diffuse hair loss, while others do not.

**Conclusion:** There may be a significant relationship between ID and alopecia areata, androgenetic alopecia, telogen effluvium, and diffuse hair loss. If ID is discovered, the cause of ID must be identified. ID can be treated safely with ferrous sulfate. Excessive iron supplementation may cause iron overload and should be avoided, especially in high-risk patients such as those with hereditary hemochromatosis. Patients who do not respond to iron replacement therapy should undergo additional testing to identify other underlying causes of ID.

**P-191**

**finasteride Treatment for Female Pattern Hair Loss: Prospective Pilot Study**

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finasteride is among the most effective FDA approved treatment male pattern hair loss. Unfortunately, it did not get the FDA approval for females because of the poor outcomes in the initial studies.

**Objectives:** To investigate for some of the reasons for poor response to finasteride in females with androgenetic alopecia through: 1, verifying the diagnosis; 2, assessment of serum level of androgens (free testosterone, DHEAS, 17 hydroxy progesterone) and 3, compliance of the patients to treatments.

**Methods:** the plan is to include 30 females with clinical diagnosis of androgenetic alopecia. Pre treatment measurements of the serum level of free testosterone, DHEAS, 17 hydroxy progesterone, standard photograph for the scalp and four mm biopsy are to be done for all patients. finasteride 1mg should be given for all patients for 24 weeks. Eight weeks post finasteride administration, serum androgen levels to be repeated with finasteride level. the later labs to be repeated after completion of 24 weeks of treatments in addition to photographs. In each patient, bimonthly counting for the given tablets are recorded.

**Result:** 14 patients have completed the 24 weeks treatment period. All of them were compliant with treatment. Scalp biopsy showed featured of androgenetic alopecia. Four patients showed good response to finasteride and another four were mildly improved. five of the 14 patients did not show any significant improvements and one got worse. Of the good responders, one found to have high androgens. There was no adverse effect in all patients.

**Conclusion:** Although not yet completed, this pilot study showed 8 of the 14 patients (57%) responded to finasteride. An amendment in the objectives was added to include the assessment of the efficacy of finasteride in female patients. By June 2007, 25 patients are expected to complete the study and we may able to show their outcomes.
The Role of the Androgen Receptor Gene CAG Repeat Polymorphism in the Development of Postmenopausal Facial Hirsutism and Body Hair Changes

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The human androgen receptor (AR) gene contains a variable number of CAG repeats. Shorter repeat length may result in augmented AR-receptor mediated sensitivity of the hair follicle and has been associated with early male balding1 and hirsutism2. We have assessed the relationship between the CAG polymorphism of the AR receptor gene and the pattern of X-inactivation of the X-chromosome in the development of postmenopausal facial hirsutism and body hair changes.

184 normal postmenopausal female of Northern European origin were examined by a single observer and assigned a facial and body hair score using a modified Ferriman and Gallwey score. Genomic DNA extracted from peripheral blood was analysed for the number of CAG repeats. X-inactivation pattern analysis was carried out by looking at the methylation status of each allele after DNA digestion with HpaII. Spearman rank correlation was used to analyse the relationship between CAG repeat length and facial and body hair scores.

Positive correlation was found between shorted CAG repeat length and facial hirsutism and skewing of X-inactivation toward the shorter allele. This was statistically significant (p<0.05) in the older postmenopausal females (age>65). No significant correlation was found with body hair.

These findings support a role for the CAG repeat polymorphism of the AR gene in the development of postmenopausal facial hirsutism and body hair changes.

P-193
p16ink4a Mediated Premature Senescence of Balding Dermal Papilla Cells

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DP cells derived from frontal (balding) human scalp hair follicles (BDPC) have been used to study Androgenetic (AGA). In this study, we have investigated the growth of human DP cells isolated from balding (BDPC) and non-balding (NBDP) male scalp. Our data confirmed previous reports that BDPC have much slower growth rates in vitro when compared to NBDP. However, we observed that the slow growth of BDPC was associated with a large flattened morphology characteristic of senescent fibroblasts and was confirmed by demonstrating expression of senescence-associated beta-galactosidase. Premature senescence was specific to BDPC and not seen in NBDCP. Also, connective tissue sheath (CTS) fibroblasts from balding follicles did not undergo premature senescence indicating that this phenomena was specific to BDPC and not due to unhealthy follicles. The senescence of BDPC was associated with terminal growth arrest as demonstrated by decreased expression of PCNA antigen and increased expression of p16INK4a. BDPC also expressed a range of markers of oxidative stress and DNA damage including HSP27, Super Oxide Dismutase, Catalase and phospho-ATM. These results demonstrate that early onset of senescence in balding DP cells is associated with up-regulation of p16INK4a possibly resulting from oxidative stress and DNA damage. Moreover, we show this is a specific characteristic of BDPC and is not seen in CTS fibroblasts from balding follicles or NBDCP. These data identify novel mechanisms that may be responsible for aspects of AGA and may be associated with induction of TGF-b by DHT in BDPC and role of TGF-b in oxidative stress.

P-194
Comparison of the Efficacy and Safety of Topical Minoxidil and Alfatradiol in the Treatment of Androgenetic Alopecia in Women

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Two drugs which are approved for the treatment of androgenetic alopecia in women in Germany were compared with regard to their influence on hair growth. Patients were randomised to group I (n = 52) using 2% minoxidil solution twice daily for 12 months or to group II (n = 51) using 0,025% alfatradiol solution once daily for 6 months and were then switched to 2% minoxidil solution for months 7-12. Changes in hair growth parameters were determined using TrichoScan.
Topical treatment with 2% minoxidil solution for 6 months resulted in a significant increase of cumulative hair thickness (p < 0.0001) and absolute hair density (p ≤ 0.0025), whereas these parameters of hair growth remained nearly unchanged after 6 months of treatment with alfatradiol solution. Evaluation of the same parameters from month 7 to month 12 demonstrated that 12 months minoxidil treatment resulted in an increasing stabilisation (group I). After the Alfatradiol/Minoxidil switch in group II a significant increase in cumulative hair thickness and absolute hair density could be achieved. The results obtained almost equalled those achieved in group I with continuous minoxidil treatment. Both study medication were tolerated very well.

Treatment with minoxidil can induce an increase in hair density and hair thickness, whereas treatment with alfatradiol results in deceleration or stabilisation of hair loss.

**P-195**

**finasteride Efficacy in Male Pattern Baldness in Iranian Men**

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**Background:** Androgenetic alopecia (AGA) is one of the most common causes of hair loss in genetically predisposed men and women. We studied the therapeutic efficacy and safety of oral finasteride in male patients with AGA.

**Materials and Methods:** A total of 120 male AGA patients, 19-55 years of age, were enrolled in the study for a period of 12 months. Oral finasteride, 1mg/day, was started and the drug efficacy was assessed on the basis of pre- and post-treatment clinical photographs, trichogram, and hair pull test by the investigators. Routine biochemical investigations and a questionnaire related to sexual disturbances were carried out to assess the safety profile of the drug at each follow-up visit every 2 months.

**Results:** Improvement in hair growth was observed by comparing the paired pre- and post-treatment global photographs (p < 0.05), hair pull test evaluation (p < 0.001) and increase in anagen-telogen hair ratio (p < 0.001) with the trichogram, as early as 4 months of finasteride therapy with maximum benefit in the middle of scalp followed by vertex and frontal areas. No significant side effect related to the drug was observed except for a little decrease in libido in 2 men.

**Conclusion:** It is concluded that oral finasteride is a relatively safe, well tolerated and efficacious drug for AGA in males.

**Key words:** finasteride, Male pattern Baldness.

**P-196**

**Trichodynia Is a Distinguishing Symptom of Telogen Effluvium**

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**Objectives:** The prevalence of trichodynia is controversial. Controversy may stem from the diagnostic confusion between androgenetic alopecia (AGA), chronic telogen effluvium (CTE) and their association (AGA+CTE).

**Approach:** With the aid of the modified wash test (WT) (1), we surveyed 10 men and 85 women complaining of hair loss. After 5-day-abstention from shampooing, they soaped and rinsed the hair in a basin and collected all hair remaining in a gauze covering the basin bottom. Hair were counted and divided into <3 cm hair (vellus hair) and into >3 cm hair. Patients with <100 total hair and >10% vellus hair were diagnosed as having AGA; those with >100 hair and <10% vellus hair were diagnosed as having CTE; those with >100 hair and >10% vellus hair as having AGA+CTE and patients with >100 hair and <10% vellus hair as having CTE in remission.

**Results:** Trichodynia was reported by 22 patients: 17 had CTE, 2 AGA and 3 CTE+AGA. None has CTE in remission. The prevalence (51%) of trichodynia in patients with CTE and CTE+AGA was statistically highly significant (c² = 20.077, p < 0.001).

**Conclusion:** Trichodynia is almost exclusive of patients with CTE as it affects about one half of the them and may be a marker of activity of an inflammatory peripilar process.

**References:**

P-197
Variability in the Androgen Receptor Gene: Strong Association With Androgenetic Alopecia, Functional Implications and Indication For Positive Selection

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Androgenetic alopecia (AGA, male pattern baldness) is the most common form of hair loss. Its pathogenesis is androgen dependent, and genetic predisposition is the major requirement for the phenotype. We have recently demonstrated that genetic variability in the androgen receptor gene (AR) is the cardinal prerequisite for the development of early-onset AGA, with an etiological fraction estimated at 0.46. The investigation of a large number of genetic variants covering the AR locus suggests that a polyglycine encoding GGN repeat in exon one is a plausible candidate for conferring the functional effect. The polyglycine tract is located in the transactivating domain of the androgen receptor protein (AR), suggesting an effect of repeat length on receptor function. We compared the functional characteristics of the two most common alleles (23 and 24 repeats) and two extreme alleles (10 and 27 repeats) in a reporter gene assay in HeLa cells. Our data provide evidence of functional differences between the two most common alleles of the AR GGN repeat. The AR haplotype with the highest frequency (0.45) in the German population, which confers risk to AGA, seems to be evolutionarily recent, as indicated by the low sequence identity with the ancestral haplotype and larger extent of haplotype homozygosity. This implies that a variant at the AR locus may have experienced recent positive selection that led to an increase in frequency of the AGA susceptibility allele in the European population.

P-198
Quantitative Analysis of the Effect of finasteride in Korean Androgenic Alopecia Patient

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Objectives: finasteride (FNS) has been widely used to treat androgenetic alopecia (AGA) in males. Most precise data to evaluating the effect of FNS was phototrichogram, but there has been relatively lack of data in Asian people. We tried to observe the effect of FNS in Korean AGA patients by using phototrichogram.

Approach: Thirty two patients with AGA were enrolled in this study. All the patients were taken phototrichogram (Folliscope, Lead M Co., Korea) before and 6 months after treatment with oral FNS (Propecia, MSD Korea, Korea) 1mg/day. We measured hair density and hair diameter only without clipping of hairs, because of the cosmetic problems.

Results: The average densities of hairs in AGA patients (104.4±26.2 /cm²) were significantly increased after 6 months treatment with FNS (120.8±25.0 /cm², p<0.01). The average diameters of hairs (60.8±13.8 mm) were also significantly increased after 6 months (68.5±14.7 mm, p<0.01).

Conclusions: We could find FNS is also effective in Asian AGA patients.

P-199
Efficacy of Adenosine on a Female Pattern Hair Loss

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Background: Adenosine up-regulates vascular endothelial growth factor and fibroblast growth factor-7 in cultured hair dermal papilla cells. We previously showed that in Japanese men, adenosine improved androgenetic alopecia due to thickening of thin hair generated by hair follicle miniaturization.

Approach: To investigate efficacy of adenosine against hair loss in women.
**Approach:** Thirty Japanese women with a female pattern hair loss were recruited in a double blind, randomized, placebo-controlled study. Volunteers topically used either 0.75% adenosine lotion or placebo lotion twice daily for 12 months. Data were obtained at 0, 6 and 12 months. Efficacy was evaluated by assessing global scalp coverage by dermatologists, and from a phototrichogram.

**Results:** After 12 months of human trial, it was shown that adenosine was significantly superior to placebo on global improvement from direct assessments by dermatologists and photograph based assessment by investigators. By phototrichogram results, adenosine lotion could show significant improvement based on the change of hair growing speed and change of thick hair rate compared to placebo. Adenosine also showed significant improvement by self-assessment results. No side effect was encountered during the trial.

**Conclusions:** Adenosine improved hair loss in Japanese women by stimulating hair growth and increasing thick hair. Adenosine is useful for treatments of androgenetic alopecia in men and a female pattern hair loss in women.

**P-200**

**Interaction of Androgen Receptor With Wnt Signaling Axis in Dermal Papilla Cells**

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**Objective:** Wnt and androgen are known to positively and negatively affect mammalian hair growth. We hypothesized that androgen reduces hair growth through the interaction with Wnt signaling system. The purpose of this study is to investigate the effect of androgen on Wnt signaling in dermal papilla cells (DPCs).

**Approach:** The effect of androgen and Wnt3a on keratinocytes (KC) proliferation was measured using coculture system of DPCs and KCs. Molecular mechanism of interaction of androgen and Wnt signals in DPCs was examined by analyzing the expression, intracellular localization and activities of androgen receptor (AR) and downstream molecules of Wnt signaling. We also studied the expression level of TGF-beta in DPCs by real-time PCR analysis.

**Results:** Wnt3a stimulated the growth of KCs when co-cultured with DPCs. Wnt3a-dependent growth of KCs was suppressed by androgen in male-derived DPCs (MDPCs) co-culture, but not in female derived DPCs co-culture. Androgen treatment suppressed Wnt-mediated transcription and promoted the expression of TGF-beta in MDPCs. While both of male- and female-derived DPCs expressed AR, the expression level and the degree of nuclear translocation of AR were higher in MDPCs.

**Conclusion:** These results strongly suggest that the inhibitory action of androgen on KC proliferation in the co-culture is mediated through the suppression of Wnt signaling as well as TGF-beta production by AR in MDPCs.

**P-202**

**Female Pattern Hair Loss Commonly Affects Sides and Back of Scalp**

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Hair thinning in female pattern hair loss (FPHL) is typically most pronounced over the top and front of the scalp. However, some women show a more widespread distribution of scalp hair loss with involvement of the sides and a back of the scalp. In this study we have evaluated the frequency of hair thinning in different regions of the scalp in a cohort of women with FPHL.

We studied 67 women with FPHL (grades 2-5 on the Sinclair scale). Using a method described by Olsen (J Am Acad Dermatol, 48;253-62; 2003) the scalp was divided into 9 regions (frontal, mid-scalp, vertex, temporal x2, lateral x2, occipital x2) and hair density was assessed in each region using a 7-point visual analogue scale).

Low hair density was most pronounced in the frontal region of the scalp, closely followed by mid-scalp and vertex. Thinning in the temporal regions was also common (70%) although none of the subjects showed deep recession of the frontal hair line. 49% of women were judged to have significant hair thinning affecting the sides of the scalp and 22% showed thinning in the occipital regions.

This study confirms the clinical impression that, unlike male balding, FPHL in women often affects the sides and back of the scalp as well as the top and frontal regions. This has implications for management of FPHL, particularly for surgical treatment, and raises further questions about the identity of the (patho)biology in pattern hair loss in men and women.
Androgenetic Alopecia: Concordance of Hair Loss in Twin Pairs

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Androgenetic alopecia is a common disorder that affects both men and women, but its mode of inheritance has been a contested topic. Our objective was to measure and compare hair loss in monozygotic and dizygotic twins and to determine concordance rates between pairs. In fraternal twins, expected concordance is 50% for a monogenetic simple Mendelian transmission and less than 50% for a polygenetic trait; 100% concordance is expected for monozygotic twins. Recruitment of twins took place at the 2004 National Twinsday Festival in Twinsburg, Ohio. Twins filled out hair questionnaires to determine demographics and exclusion criteria. Each participant had stereotactic frontal/vertex/occipital scalp photographs (Canfield Scientific). Buccal swabs were collected for zygosity testing and were performed by AnaGen Technologies, Inc. The photographs were evaluated in a blind manner by one of the investigators (VHP) for degree of alopecia and concordance/discordance amongst twin pairs. A total of 44 twin pairs were enrolled, 16 were excluded from the study. Of the 28 twin pairs evaluated, 2 of the 4 dizygotic twins were discordant (50%) and 3 of the 24 monozygotic twins were discordant (11%). This preliminary data shows an unexpected discordance rate of 11% amongst the monozygotic twin pairs suggesting that environment may have a greater influence on androgenetic alopecia than previously recognized. Ongoing enrollment of twins in the study will allow for larger sample size and improved power to further evaluate the inheritance pattern of androgenetic alopecia.

A Multicenter, Randomized, Placebo-Controlled Double-Blind Clinical Trial of a Novel Formulation of 5% Topical Minoxidil Foam vs. Placebo in the Treatment of Androgenetic Alopecia in Men

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Although 5% topical minoxidil solution is safe and effective, a vehicle that does not contain propylene glycol and is more aesthetically pleasing to the consumer, would be a distinct advantage to consumers for use in androgenetic alopecia (AGA).

Objective: To assess the efficacy and safety of 5% topical minoxidil when formulated in a new foam vehicle (TMF) for men with AGA.

Method: Two-phase study:

- Sixteen week double-blind placebo-controlled phase to evaluate the efficacy and safety of the 5% TMF. This phase was conducted on 352 men ages 20-49 with patterns IIIv, IV or V Hamilton Norwood with the primary efficacy endpoints of change between Baseline and Week 16 target area hair counts (TAHC) and Week 16 subject assessment of change in hair loss condition from Baseline.
- Open-label extension phase to collect 52 weeks of safety data with 5% TMF. One hundred forty-three subjects continued on this phase of the study. Safety was monitored by taking intercurrent history, vital signs and scalp irritation assessment by both investigator and subject.

Results:

- Statistically significant increase at Week 16 compared to Baseline in TAHC with the 5% TMF group (170.8 to 190.8 hairs) compared to placebo (168.9 to 174.4) (p<0.0001).
- Statistically significant subjective assessment of hair loss condition (p<0.0001) on 5% TMF (70.6% noted increased hair growth, including 47.8% moderate or marked hair growth) compared to placebo (42.4% noted increased hair growth, including 21.5% moderate or marked hair growth).
- No significant safety concerns were raised and the 5% TMF was well tolerated over a one year use period.

Conclusions: The 5% topical minoxidil product, formulated without propylene glycol and in a foam vehicle, is a safe and effective treatment for men with AGA.
**P-205**


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**Objective:** The purpose of this study was to evaluate the family history, and clinical status of patients with androgenetic alopecia.

**Approaches:** 789 patients with androgenetic alopecia were assessed at the Alopecia Clinic, Department of Dermatology, College of Medicine, ChungAng University Hospital over a 3 year period from 2004 to 2006

**Results:**
1) Men (520 patients) are affected 1.9 times more than are women (269 patients). Most of them are twenties (male 223; 42.8%, female 85; 31.5%). 1.9:1.0.
2) In the 520 male patients, Norwood class IIIv was dominant (161 patients; 30.9%). In the 269 female patients, Ludwig class I was superior (218 patients; 81%).
3) 395 patients (75.9%) of 520 male patients and 198 (73.6%) of 269 female patients had a family history.
4) The most common accompanying disorder was seborrheic dermatitis (male 407; 78.2%, female 155; 57.6%). And others include atopic dermatitis, hypertension, thyroid disease, etc.
5) Serum testosterone level were increased in 92 (17.6%) of 520 male patients and 36 (13.3%) of 269 female patients.

**Conclusion:** Most of these results are compatible with our previous study carried out in 2004. But, female androgenetic alopecia patients are nowadays increasing in number.

**P-206**

**Men with Kennedy's Disease Have a Reduced Risk of Androgenetic Alopecia**

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**Background:** Twin studies have demonstrated male pattern hair loss (MPHL) has a hereditability of around 80%, indicating that around 80% of the total variance in MPHL could be attributable to additive genetic effects. The heritability of female pattern hair loss (FPHL) is not known, nor is the hereditability of dandruff or greying of hair.

**Objectives:** To investigate the heritability of FPHL through twin concordance.

**Methods:** A pilot study was conducted among 100 twin pairs. All completed questionnaires prior to attending scalp examination. Subsequently questionnaires were mailed to 1000 female twin pairs (2000 females), aged between 21 and 75. Twins recorded their own hair thickness and that of their sister as well as presence or history of dandruff, grey hair and cancer.

**Results:** 412 (=824 QN) questionnaires (response rate 41.2%) were returned, of which 249 were monozygotic (N=498) and 163 were dizygotic (N=326) twin pairs. Heritability for FPHL in the pilot study was 0.75. In the main study heredability was ~0.69 in self-scored hair thickness and ~0.86 in twin rating hair-thickness.

**Conclusion:** There is a strong heritability for female pattern hair loss, under both conditions of self-rating of hair thickness, and twin rating of hair thickness. Additive genetic effects play a major role in the development of FPHL.
P-208
Uncommon Tropical Hair Disorders
Sundaram, Murugusundram; Yesudian, Patrick; Skin Hair & Nail Clinic, Chennai, India

Uncommon scalp disorders like pityriasis amiantacea and plica polonica are frequently seen in southern parts of India. We present ten such cases of cosmetic significance.

Many female patients and few male patients of ages varying from 20 to 50 presented with matting of hair and thick white scales adhered to the hair to our clinic. All were asymptomatic except for occasional itching. All were concerned about the unpleasent appearance of the matted hair.

Hair examination was done in all the cases to find out the presence of parasitic infestations, fungal infections and casts. Scalp biopsy was done in a few unresponsive cases to rule out psoriasis. A detailed history was taken to reveal the hair grooming habits.

No cause was found in most of the cases. Majority of them showed pityrosporum folliculitis. One had psoriasis. Two had parasites and fungus. Many of them responded to karyol shampoo. We advise tonsuring of hair in a few. Psoriasis was treated with coal tar shampoo and topical steroids.

These disorders pose a significant cosmetic problem. They are mainly due to the tropical weather and humidity coupled with infrequent and inadequate hair cleaning habits. In certain parts of south India these are due to religious practices among the head priests who grow long hair.

P-209
Peculiar Forms of Pressure Alopecia
Sundaram, Murugusundram; Yesudian, Patrick; Skin Hair & Nail Clinic, Chennai, India

Alopecia occurring at sites of constant pressure is common. We present a few peculiar rare forms of pressure alopecia.

Occipital pressure alopecia is very common in infants in south India because if the habit of sleeping on the floor. Pony tail alopecia common in female children is not very uncommon in the male children of south India because hair is grown lengthy in male children also till it is offered to God as a religious custom. A form of pressure alopecia is common in male sikh children because if the tight hair style forced by the the sikh religious custom. Pressure alopecia is also a common place affair in hindu priests who grow long hair and tie tightly a tuft if hair. Spectacle frame alopecia is common in young adults because of the tight spectacles frames worn in order to prevent the spectacles falling due to the slippery sweat common in the humid tropical weather. Hair pieces now being commonly used give raise to pressure alopecia due to the clips. Oil massage of the scalp which is very popular in various parts of south India advocates vigorous rubbing of hot oil over the scalp causing pressure alopecia sometimes irreversible.

These peculiar forms of pressure alopecia are unique and common in south India. This paper is to emphasize the cultural, social geographical, environmental and religious influences in studying clinical hair disorders.

P-210
Posterior Parting: a Necessary Photo Control For Female Pattern Hair Loss Diagnosis and Evaluation
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Objectives: Evaluate “posterior parting” besides the anterior parting width as a complementary and effective method for Female Pattern Hair Loss (FPHL) diagnosis and documentation.

Approach: Dr. Elise Olsen has described the “frontal parting” as an effective photographic control for patients with FPHL. It is a useful technique, and we also consider the evaluation of the posterior parting for scalp documentation, helping with the differential diagnosis of chronic telogen effluvium and diffuse hair loss. In a photo studio, with a professional digital camera, we proceed the standard photos – frontal, both diagonal and top view, plus the frontal parting, with the patient forehead rested over a cephalo-static device. With a regular comb and clips we expose the posterior midline from top to bottom to perform the “posterior parting” photo.

Results: The main advantage of anterior parting is to estimate the amount of hair thinning and loss of density, which are not specific of FPHL. Comparing frontal to posterior parting we can: determinate the extension of hair thinning and realize if the process is more diffuse or localized (providing an idea of the original hair density). Medical treatment options for FPHL are limited and hair restoration surgery should be considered as a complement to hair treatments.

Posterior parting is an extremely valuable technique, allowing an estimative of donor area density (occipital) and its viability for performing a hair restoration.

Conclusion: Posterior parting photo control is an effective method not only for FPHL evaluation and diagnosis, but also helps estimating the donor area density for a possible hair restoration surgery.
P-211

Retrospective Study Into the Effect of finasteride on Hair Thickness in Male and Female Patients With Androgenetic Alopecia

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Between 2003 and 2006 over 2000 patients consulted the trichologists of a specialized Dutch clinic. finasteride was prescribed to men and women, diagnosed with androgenetic alopecia. Prescription of finasteride to women was restricted to Post-menopausal women and women who stated in writing that they were not pregnant at the time of prescription and would not become pregnant during the treatment. Individuals took 1.25 mg of finasteride daily and compliance was monitored by a count of the prescribed medication. At baseline and at one and two years after baseline, reproducible images of the head and hair were made. In addition, detailed images of four predilection sites for androgenetic alopecia were analyzed for hair density and thickness. The effect measure for finasteride treatment consisted of the thin-to-thick (TT) ratios of four sites at the scalp. To determine a TT-ratio, the sum of the thickness of the three thinnest hairs was divided by that of the three thickest hairs.

876 compliant subjects were remeasured after one year, and 364 subject were measured two years after baseline. The overall TT-ratio’s in all subjects at baseline for four sites at the midline (front, center, vertex and rear) was 0.25 (SD 0.05). In both the male and female subjects the TT-ratio was the same (0.25; SD 0.05). One year after baseline, the overall TT-ratio was 0.34 (SD 0.07). The ratios for male and female subjects were both 0.34 (SD 0.07). Two years after baseline, the overall TT-ratio was 0.37 (SD 0.07), whereas both ratio’s for male and female subjects were 0.37 as well (SD 0.08). The TT-ratio’s of male and female subjects, both one and two years after baseline, were not statistically significantly different (Student t-test; P<0.01).

The results of this study indicate that finasteride is effective in establishing thicker hair in men and women.

P-212

Estimation of finasteride Sensitivity of Male Pattern Baldness Patients By Determination of Triplet Repeat Number in Androgen Receptor Gene and Hormone/Cytokine in Serum

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Objectives: finasteride is effective on male pattern baldness (MPB) although there is a variation in the efficacy of this drug among the MPB patients. To know any factor, which correlates with the effectiveness of finasteride, the polymorphism of androgen receptor (AR) gene and serum levels of certain hormones and cytokines were analyzed.

Approach: After PCR of blood cell DNA, number of triplet repeats (CAG+GGC) in the first exon of AR gene was determined. Testosterone, dihydrotestosterone, IGF-1 and TGFb-1 were determined by immunological assays before and one month after the drug treatment. Symptoms, typed by photographic method before and 6 months after the drug treatment, were compared.

Results: When the number of the triplet repeats was plotted against the degree of symptom improvement after treatment, a broad correlation between these variables was observed. The smaller the repeat number, the higher the improvement. finasteride was more effective on the improvement of patient group with smaller repeat number in AR gene@(<40) than that with larger one (>41). There was a unique tendency in initial level of hormone and cytokine in the smaller repeat number group. Furthermore, change in these factors after treatment with finasteride for one month was also characteristic. On the other hand, no such inclination was observed in larger repeat number group, who were poor responder to finasteride.

Conclusion: Effect of finasteride after a long-term (6M) treatment on individual patient is able to be estimated by determinations of AR gene polymorphism and survey of hormone/cytokine levels within a short-term (1M).
**P-213**  
The Estrogen Receptor Beta Gene, ESR2, in Female Pattern Hair Loss  

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**Objectives:** Female pattern hair loss (FPHL) is a complex polygenic trait with an unproven relationship with androgens. The genes involved in the pathogenesis of FPHL remain unknown, but are likely to include genes related to the androgen and estrogen pathways. The ESR2 gene is located on chromosome 14q22-24 and codes for the estrogen receptor beta (ER-b). Estrogens play a vital role in non-reproductive tissues in both sexes and are able to modify androgen metabolism in hair follicles. In contrast to ER-a, ER-b is the predominant subtype within the outer root sheath and epithelial matrix, making it likely that the ESR2 gene directly modulates the hair growth cycle. This study aims to evaluate the relationship between ESR2 gene variants and FPHL.  

**Approach:** Allele and genotype frequencies of tag single nucleotide polymorphisms (tag SNPs) in the ESR2 gene were compared between 150 cases with FPHL (stage 3 or greater on the midline clinical grading scale), and 90 controls (aged >50 years with no hair loss conditions), using c²-tests. Tag SNPs were chosen using the HapMap database, and are representative variants in a region of linkage disequilibrium, whereby their examination would be sufficient to capture the genotypic information of all known SNPs in that region.  

**Results:** Three ESR2 tag SNPs that collectively capture 33 SNPs in the gene have been analysed and there were no significant differences in allele or genotype frequency between cases and controls (p>0.1).  

**Conclusion:** By essentially examining 33 SNPs located in the ESR2 gene region, we have been unable to identify genetic association between ESR2 and FPHL. However, the association of this gene with FPHL cannot yet be discounted. We are currently analyzing the remaining tag SNPs necessary to capture all known SNPs in the gene region, and are recruiting an additional 275 cases and 400 controls through the Melbourne Collaborative Cohort Study to increase statistical power of this study.

**P-214**  
Comparison of Microscopic Images of Animal Vibrissae Hair Shaft Using Hard X-Ray Microscopy  

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**Objectives:** Recently, it was introduced that microscope using highly coherent, bright third generation x-ray could be an alternative way to visualized the ultrastructure of material. The hard x-ray from the third generation synchrotron light source has a higher energy with better penetration and there is no needs to sample preparation so it is more suitable for the biological samples(ex. hair) without processing artifacts or damage to samples.  

**Approach:** We firstly investigated the ultrastructure of some mammalian vibrissae hair shafts, including dog, cat, rabbit, rat, mouse, and hamster with hard X-ray microscopy in Pohang Accelerator laboratory.  

**Results:** Three distinct structures (medulla, cortex, and cuticular layer) of vibrissae hair shaft were clearly distinguished in all species except cat. We also could find that the detailed characters of each sample were different. Among them, the most distinct characteristics is the medullar structures.  

**Conclusion:** We firstly visualize the integrated internal structures of various vibrissae hair shaft with this new technique.

**P-215**  
A Rare Family Case of Moniletrix  

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Moniletrix is a rare hair disease generally inherited with an autosomal dominant fashion. Walter Smith in Dublin first described the disease in 1879 as “rare nodose condition of the hair.” Successively, Radcliff Cocker named the disease moniletrix to define a clinical condition characterized by extreme fragility of the hair which appeared rosary beads shaped. Hair shaft is beaded and shows elliptic nodes with a diameter ranging from 0,7mm to 1,0mm, separated by narrower internodes. Hair easily breaks and is not able to achieve a normal length.
The Authors present a case of a family composed of six members. Four of them were affected with the rare disease. In particular, they reported the striking healing obtained by treating the youngest daughter with griseofulvin.

P-216
Hard x-Ray Microscope; New Investigative Tool For the Hair Morphology

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Objectives: Better visualization of morphologic details of hair fiber has great importance in the fields of cosmeceuticals, clinical diagnostics, and so on. Conventional methods to investigate hair morphology including light or electron microscope have limitations in terms of limited resolution and laborious sample processing.

Approach: Hard x-ray microscope uses bright, coherent and highly collimated x-ray from the 3rd generation synchrotron as a light source. X-ray from the synchrotron was attenuated by hair fiber and converted to visual light at the scintillator. To visualize hair fiber more clearly, phase contrast lens was applied. Resolution power was about 90nm. Sample hairs obtained from various races and body sites were just mounted without any processings.

Results: Images revealed natural characteristics of hair fibers. Detailed structure of cuticle, cortex and medulla was clearly visualized. Several overlapped cuticle, vertically oriented macrofibrils of the cortex was distinguished. Medulla showed amorphous granular structures.

Conclusion: Hard x-ray microscope is useful tool to investigate detailed natural morphologic characteristics of hair fiber.

P-217
Ultrastructure of Fine Merino Wool

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Merino wool is a fine high-curl fibre that is often used as a model system for understanding the behaviour of other mammalian fibres such as human hair. The structural elements of a single wool fibre that define its mechanical properties occur across a wide spatial scale. Both large-scale features, for example the distribution of multiple cells of different types, and small-scale features, for example the arrangement of intermediate filaments, are implicated in defining the wool fibre's structural integrity. We have constructed a series of scale illustrations at the whole-fibre, cellular and sub cellular scales for Merino wool which summarise the results of our recently completed survey of Merino wool ultrastructure, the most comprehensive to date.

P-218
Comparison of Hair Shaft Damages By Different Spectrums of Ultraviolet Light

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Objectives: Among the various causes of extrinsic hair damages, exposure to sunlight, especially ultraviolet light is inescapable in daily life. Interests about photoaging in skin have been increasing for many decades, but studies and concerns about the effects of ultraviolet light to hair have been emerging recently. Macroscopically, hairs exposed to ultraviolet light tend to be dry, course and stiff, and usually lose their strength, color and luster. In this study, we investigated the patterns of microscopic and chemical changes of photo-damaged hairs after irradiating different spectrums of ultraviolet light.

Approach: To observe the patterns of changes caused by different spectrums of ultraviolet light, scanning and transmission electron-microscopy, and protein analysis method to detect soluble hair protein were employed.

Results: Structural changes such as hair cuticle damages were induced mainly after UVB irradiation, while hair soluble protein changes were induced mainly after UVA irradiation. UVB and narrow UVB showed similar results.

Conclusion: Different spectrums of ultraviolet light may cause different patterns of damage to hair shaft.

P-219
The Form of Hair Follicle Determines the Shape of Hair

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Objective: In order to investigate the effects of the form of hair follicle on the decision on the shape of hair shaft we analyzed the correlation between the shape of hair shaft and those of hair follicle by grafting the isolated hair follicles from newborn rat vibrissa onto the back skin of severe combined immunodeficient (SCID) mouse.
Approach: The shape of regenerated hairs was observed. Some hairs were plucked to examine the shapes of the hair in the next hair cycle. HE staining was also proceeded to study the shape of hair follicles after regeneration.

Results: Almost all grafted hairs were regenerated on the back of SCID mice. About sixty percent of regenerated hairs showed straight shape and the rest of hairs became bent even though only straight hair follicles were grafted. Plucked hairs were discovered to keep the same hair shape. HE staining of regenerated hair follicles demonstrated that straight hair shape was derived from straight hair follicle and curved bulb region resulted in wavy hair shape. In addition, kinky hair shape was found to have remarkable bent hair follicle in total.

Conclusion: Taken together, these findings strongly suggest that changes of degree in bending hair follicles result in various shapes of hair in proportion, indicating a prominent correlation between the shape of hair shafts of those of hair follicles.

P-220
Implanted Hair Follicle Stem Cells Facilitate the Repair of Severed Peripheral Nerves and the Spinal Cord
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The hair follicle bulge area is an abundant, easily accessible source of actively growing, pluripotent adult stem cells. The fluorescent protein GFP was used to follow the follicle stem cell's fate. The pluripotent hair follicle stem cells are positive for the stem cell markers CD34 and nestin but are negative for the keratinocyte marker keratin 15, suggesting their relatively undifferentiated state. The hair follicle stem cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. In vivo, the hair follicle stem cells greatly enhance the rate of nerve regeneration and the restoration of nerve function. After transplantation in the severed nerve in mice, the hair follicle cells transdifferentiate largely into Schwann cells, which are known to support neuron regrowth. Function of the rejoined sciatic nerve was measured by contraction of the gastrocnemius muscle upon electrical stimulation. After transplantation of GFP-expressing hair follicle stem cells between the severed thoracic region of the spinal cord of mice, the hair follicle stem cells also differentiated into Schwann cells and joined the severed spinal cord. The rejoined spinal cord improved hind-limb locomotor performance. Thus, hair follicle stem cells provide an effective accessible, autologous source of stem cells for treatment of peripheral nerve and spinal cord injury.

P-221
Towards Potential Markers For the Human Hair Follicle Stem Cell Niches
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Regeneration of wounded skin has been attributed to hair follicle stem cells (HFSC). To utilize HFSC as a source for the generation of human autologous skin transplants, explicit characterization of these cell populations and their particular niches is desperately required. The actual approach therefore aims in the definition of potential markers for the multipotent epithelial cell population of the human HF in correlation to complementary precursor cells of mesenchymal origin.

For this purpose, skin biopsies obtained from excess of surgeries were processed to cryo-sections with longitudinally orientated HF and stained by immunofluorescence.

Cytokeratin 15, recently identified as one preferential marker of the human epithelial HF stem cell region, was co-visualized with Vimentin, a marker for mesenchymal derived fibroblasts. Cytokeratin 15 immunoreactivity (IR) was expressed in keratinocytes of the distal and central outer root sheath (ORS) including the bulge region, adjacent to isolated Vimentin IR fibroblasts of the connective tissue sheath. In addition, isolated fibroblasts of the upper proximal HF as well as clusters of fibroblasts in the lower bulb and the HF dermal papilla were Vimentin IR.

Tenascin C and further candidate marker Collagen IV, showed IR along the peripheral connected tissue, demonstrating their usefulness in demarcating stem cell compartments. Other tested markers such as Desmin and Collagen XVIII did not show designated IR expression.

These results suggest a crosstalk between epithelial and mesenchymal precursor cells and demonstrate an increasing significance in defining the HF stem cell niches by simultaneous co-visualization techniques.

P-222
Immunohistological Demarcation of the Human Hair Follicle Bulge Region: Indications of Immune Privilege, and Expression of Potential Stem Cell-Niche Elements
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This immunohistological study aimed at obtaining evidence on whether or not the bulge region of the outer root sheath (ORS) of normal human anagen hair follicles is likely to show relative immune privilege (IP), and at examining the usefulness of selected antigens as markers for the human
bulge region, its stem cells and/or its niche characteristics \textit{in situ}.

MHC class Ia and class II antigen expression was relatively downregulated in the human bulge, compared to the more distally located ORS. Instead, immunoreactivity for CD200 receptors and locally generated potent immunosuppressants like TGFβ1 and aMSH were upregulated in the bulge. Immunoreactivity for keratin15, keratin19, tenascinC, fibronectin, fibrillin2 and the transcription factor Lhx2 was found to be upregulated in the human bulge. In contrast, b1integrin, a6integrin, nidogen and LTBP1, showed no increased immunoreactivity here. For connexin43 and CD34 no bulge immunoreactivity was seen, while connexin43 was prominently expressed in the stratum spinosum of the epidermis and the ORS of the distal part of the HF and CD34 in the outermost layer of the ORS beyond the bulge. Consistent with previous reports, these two antigens therefore, may be exploited as useful negative human bulge markers \textit{in situ}.

In summary, the bulge may indeed represent an additional area of relative IP in human skin (though to a lesser extent than the anagen hair bulb). The therapeutic restoration and protection of this bulge IP may be a sensible strategy in cicatricial alopecia management. These IP markers are welcome additions to a defined panel of useful positive and negative markers that help to demarcate the human bulge region and its niche characteristics \textit{in situ}.

P-223

\textbf{Inner Root Sheath Morphology in the Histology of Trichotillomania}

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Trichotillomania is a condition typified by self-manipulation of hair, usually of the scalp. This results in localized or diffuse alopecia producing a clinical picture characterized by broken hairs of varying lengths or absent hairs. Histologic clues to trichotillomania that may be present to varying degrees include completely or partially avulsed or torn-away hairs, perifollicular hemorrhage, twisted follicles, pigment casts, trichomalacia, overlying lichen simplex chronicus, and increased catagen and telogen follicles. Herein we describe another histologic clue to the diagnosis of trichotillomania. Normally the inner root sheath (IRS) is a round or oval shape, but in the context of torn-away hairs (shafts that are ripped from the follicle, leaving the inner and outer root sheaths behind), the inner root sheath collapses inward, assuming geometric configurations that include in decreasing frequency: [1] linear (flattened), [2] triangular, [3] round, and less likely a [5] square shape. The force of the dermis and outer root sheath pressing on the IRS produce these shapes, since the IRS lacks support internally after the hair shaft is removed by the patient. The probability of finding a particular shape is determined by the stability of the configuration, with linear (flattened) being the most stable (and therefore the most prevalent), followed by triangular, then round, and finally a square morphology. Observation of these geometric IRS configurations is highly suggestive of trichotillomania and is a useful histologic clue to the diagnosis.

P-224

\textbf{Aging of Hair I: Hair Properties and Structure}

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The effects of aging on hair thinning and on hair graying are well known, but the change of other hair properties and microstructure with age has, so far, not been clarified. Therefore, we have systematically investigated the change in hair properties and hair microstructure as a function of age. 230 panelists of Japanese women aged from 10 to 70 years old were randomly selected. Hair luster and hair bounce were evaluated by both hair stylists and hair researchers. Around 100 hair fibers were sampled from each panelist to analyze the shape, physical properties and microstructure of hair.

We have found that lustrous hair is frequent in young women but not in older women. The shape of lustrous hairs was relatively straight and fibers were well aligned, while the shape of lusterless hairs was often curved and fiber alignment was disordered. The mean curl radius of hair also decreased with age. These results suggest that the increase in curved hair with age causes the decrease in hair luster. Furthermore, the microstructure of hair was investigated by synchrotron microbeam X-ray. Higher structural inhomogeneity was found in the curved hair.

In addition, it was confirmed that hair bounce (bending stress of the hair fiber) decreases after 40 years of age. Although the bending stress depends on both hair thickness and elasticity, it was found that only hair thickness decreases with age while the hair elasticity does not decrease.
The Quality of Life Implications of Androgenetic Alopecia and the Importance of Helping Patients Deal with its Psychological Effects

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Androgenetic alopecia often affects an individual’s physical attractiveness, body image, and general psychological state. In particular, it can cause anxiety, depression, psychosocial problems, personality disorders, and an inability to cope.

To help quantify how specific quality of life (QoL) issues impact patients experiencing hair loss, a questionnaire, called the Kingsley Alopecia Profile (KAP) was produced. The KAP, a valid and reliable measure, was developed following interviews with participants suffering from androgenetic alopecia and after evaluating the literature and seeking expert views.

This study showed that hair loss sufferers had increased anxiety and depression, and reduced self-esteem and social interaction. Younger and/or single patients were more impacted. The clinical picture of the condition did not correlate with QoL effects. Furthermore, the results of groups with hair loss who were not concerned (called “hair loss contented”) compared favorably with groups with no hair loss (“normals”). The results of groups without hair loss who believed they had the condition (“hair dysmorphic”) were similar to patients with hair loss who were concerned about their condition (“motivated”).

It is important to help patients deal with psychological issues as part of an overall treatment strategy for hair loss. If time is a limiting factor, it is advisable to either have trained staff to counsel and advise a patient, or to use a practitioner that specializes in this field. It is also important to help the patient be pro-active with relevant treatment, lifestyle, and cosmetic advice.

Control of Oxidative Hair Fiber Damage from Permanent Colorant Products

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Background: Use of permanent hair colorants is widespread and allows the consumer to either change their natural hair color and/or cover gray. However, one of the main trade-offs of coloring is the fiber damage that is sometimes seen over multiple uses.

We have discovered that the uncontrolled production of free radicals during the coloring process can be a significant contributor to fiber damage. We will share two key strategies that we have utilised to control the reactivity of the radical species.

Controlling Radical Reactivity during Hair Coloring

Two key reactive species in the colouring process have been shown to be responsible for the majority of the fiber damage. These two species are the perhydroxyl anion (HOO⁻) and the hydroxyl radical (HO•). The hydroxyl radical is formed from the activation of hydrogen peroxide by a transition metal capable of readily undergoing 1-electron oxidation such as copper.

(1) Chelation

We have demonstrated that the addition of a chelant to the hair colourant such as N,N’-ethylenediamine disuccinic acid (EDDS) can significantly reduce the formation of the hydroxyl radical. We have also shown that the choice of chelant is crucial. In particular, the chelant must have a high selectivity for complexing to copper, especially in relation to other metals such as calcium that are commonly found in hair.

(2) Radical Scavengers

The purpose of a radical scavenger is to trap a highly reactive radical rapidly converting it into a less reactive species. We have demonstrated the effectiveness of adding a range of radical scavengers to hair colorants. We have shown that when formulated to provide equivalent levels of bleaching (dL), the addition of a radical scavenger such as glycine, glutamine, glucosamine, arginine or proline can reduce the fiber damage significantly.

Childhood Trichotill Vs. Trichotillomania

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The diagnosis of trichotillomania (TTM) is reserved for persons who recurrently pull out their hair, experience increased tension before and gratification after the act, and have significant occupational or social impairment as a result. We propose the term trichotill (TT) to describe children with a hair-pulling habit not associated with psychological impairment and which resolves with time. We report our observations of 13 children with hair-pulling behavior. Eight children had TT whereby hair-pulling resolved in seven cases without psychiatric intervention. Precipitating factors were noted in 4 of the 8 cases. TT is a habit disorder which does not fulfill diagnostic criteria for TTM.
P-228
Aging of Hair III: The Anti-Aging Efficacy of Eucalyptus Extracts

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Older women have the complaint of hair bounce reduction because of hair thinning with age. For the complaint, there are many types of hair growth enhancer, which increases hair density and/or hair thickness. On the other hand, older women also have the complaint of decrease in hair luster with age. We have found that curved hair increases with age and the curved hair causes the hair luster decrease. Furthermore, we have found that Eucalyptus extract can improve not only hair bounce but also hair luster of older women. In this study, we studied the mechanism of Eucalyptus extract on hair properties.

A scalp lotion containing 3% of Eucalyptus extract was prepared. The lotion was applied to the scalp of female panelists (aged 40-60), who had the complaint of hair bounce and hair luster. The lotion was applied twice per day for three to twelve months. Hair fibers were sampled before and after the application and used for the analyses of hair properties. Hair luster and bounce were evaluated by hair stylists. Hair curvature and hair elasticity were measured.

We have confirmed that the hair luster and bounce of older women are improved by the application of Eucalyptus extract. It was found that hair curvature is reduced and hair elasticity is raised by the application. These results suggest that Eucalyptus extract has an anti-aging efficacy in terms of hair shape and bounce.

P-229
The Role of Laminin-332 and -511 in the Regulation of Hair Cycling and Chemotherapy-Induced Alopecia Models

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Objectives: In order to assess the role of laminins on the hair cycle and on the induction of chemotherapy-induced alopecia.

Approach: The expressions of laminin-332 and -511 and their binding partners, a6b4 and a3b1 integrins, were assessed by real time RT-PCR, immunoblot, in situ hybridization and immunohistochemistry using murine hair cycling model and cyclophosphamide-induced alopecia model. Moreover, functional aspects were assessed using recombinant laminin-332 and -511 on human hair culture.

Results: (1) In the anagen, laminin-332 and a6b4 integrin were once upregulated in anagen I-III, then became downregulated and diminished after anagen VI in the BM around hair bulbs. In contrast, laminin-511 and a3b1 integrin became constantly upregulated throughout each anagen stages. The functional study suggested that laminin-511 had a positive effect on the hair growth and laminin-322 suppressed such an effect.

(2) In the catagen, the expressions of laminin-332 and a6b4 integrin were gradually recovered in the BM around hair bulbs. Intriguingly, strong expressions were observed in the epithelial strand (ES) and connective tissue sheath (CTS).

(3) In the dystrophic catagen caused by cyclophosphamide, the expressions of laminin-332 and a6b4 integrin were strikingly upregulated in the BM around hair bulbs. They were more marked in the ES and CTS. In contrast, the expressions of laminin-511 and a3b1 integrin in the BM around hair follicles were gradually depressed.

Conclusion: The laminin-511 and a3b1 integrin positively regulate hair cycle. The laminin-332-a6b4 integrin suppressed it. Rapid disruption of such interactions possibly induces dystrophic catagen caused by the chemotherapy.

P-230
North American Hair Research Society Mentorship Program

Hickman, Janet G.;1 Ducic, Madeleine;2 McMichael, Amy;3 Elston, Dirk;4 Roberts, Janet L.;5 Sundberg, John P.;6
1. Dermatology Consultants, Inc., Lynchburg, VA, USA; 2. MD Anderson Cancer Center, Houston, TX, USA; 3. Wake Forest University, Winston-Salem, NC, USA; 4. Geisinger Medical Center, Danville, PA, USA; 5. Northwest Dermatology and Research Center, Portland, OR, USA; 6. The Jackson Laboratory, Bar Harbor, ME, USA

Grants for up to $3000 each are available annually to scientists in training, dermatology or dermatopathology residents, fellows, or junior faculty to enable study with an expert in hair research or clinical hair disorders. Either the mentor or the applicant must be a member of the North American Hair Research Society. The goal of this program is to foster the careers of young physicians, veterinarians, and basic scientists interested in hair biology or hair disorders by acquiring additional research or clinical skills, thereby furthering their careers as leaders in the field of hair research. The focus of the program is on establishing mentoring relationships. In the 5 years of the program, we have funded over 30 individual mentorship grants for...
recipients to work on projects ranging from learning specific clinical procedures to mapping mutated genes responsible for a variety of hair diseases. The short applications are due 31 December each year with funding beginning in mid-February.

**P-231**

**Aging of Hair II: Gene Expression Pattern of Vascular Endothelial Growth Factor in Hair Root and Relation to Hair Properties**

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Loss of hair elasticity by aging is one of the serious troubles in men and women. It is assumed that this physical change of the hair shaft is caused by metabolic changes in hair root cells. Then, we investigated into the factors which closely related to the hair elasticity.

Sixty-six healthy female volunteers aged between 11 and 70 years participated in this study. Approximately fifty anagen hairs were plucked from each participant's scalp. Transcriptional profiles in hair root cells of the plucked hair were examined by DNA microarray and quantitative RT-PCR. Hair properties such as diameter, elasticity and luster were also measured from the plucked hair shaft. Next, female volunteers with elastic hair (n=15) and with no or less elastic hair (n=15) were recruited, and the transcriptional profiles in hair root cells and the hair properties were examined similarly.

From among the multiple gene expressions found in hair root cells, we focused on vascular endothelial growth factor (VEGF). The VEGF gene expression level in hair root cells was the highest in teens, and thereafter declined with age. In addition, the VEGF gene expression level in participants with elastic hair was significantly higher than that in participants with no or less elastic hair. A positive correlation was determined between the amount of VEGF mRNA and the bending elasticity value of the hair shaft.

These results suggest that the VEGF in hair root cells might be a factor contributing to the maintenance of the hair elasticity.

**P-232**

**Comparison of Hair Follicle Forming Properties Between Dermal Papilla and Dermal Sheath**

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Objectives: Epithelium-mesenchymal interaction is essential component in the formation of hair follicles. The mesenchymal components, both dermal papilla(DP) and dermal sheath(DS), showed hair follicle forming, in other word, trichogenic properties but their optimal features were not revealed yet. So we investigate the differences of the trichogenic property of the DP and DS.

**Approach:** DP, upper and lower DS cells were dissected by surgically or enzymatically from the wild type vibrissae. Cells from the culture with low passage or fresh dissected were implanted to mouse ear or foot pad. The implantation depth was controlled by upper, mid or lower dermis. Reconstituted hair follicles were compared and histologic observation was performed.

**Result:** Cells from the DP and lower DS, when implanted in the upper dermis, showed similar trichogenic properties but upper DS did not induce hair follicles in any depth. Surgically dissected DP or DS cells were more apt to hair formation than enzymatically digested one but the results were not consistent. Fresh dissected DP or lower DS also showed hair follicle formation but yield was low.

**Conclusion:** Trichogenic property of mesenchymal cells, DP and lower DS, is well maintained when these cells were surgically dissected, cultured with low passage, and implanted in upper dermis.

**P-233**

**Differential Expressions of Biomarkers in Human Interfollicular Keratinocytes In Situ and In Vitro**

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**Introduction:** Mammalian interfollicular keratinocytes (IFKC) have differentiation capacity into hair follicles and a unique expression profile of cell surface markers and cytokeratins, which are distinct from epidermal keratinocytes (EKC). To characterize human IFKC, we investigated and compared expression patterns of biomarkers between IFKC and EKC, both in situ and in vitro.

**Materials and Methods:** Immunohistochemistry (IHC) of human scalp skin and hair follicles for biomarkers previously reported as specific for IFKC was performed. Expressions of the biomarkers in freshly-isolated and cultured IFKC and EKC were also investigated by FACS analysis and immunocytochemistry (ICC).

**Results:** Keratin 15 (K15) was expressed in the bulge area in IHC. CD34 was expressed in the lower area, which includes the sub-bulge area and outer root sheath (ORS). CD271 (p75 neurotrophin receptor) was expressed in the lower ORS that was negative for K15. All of them were expressed only in the outermost layer adhering to
the basement membrane. ICC of cultured IFKC revealed transitory expression of K15 in passage 1, but not of CD34 and CD271. ICC of cultured EKC showed similar expression pattern to cultured IFKC. However, FACS analysis showed prolonged expressions of K15 and CD34 up to passage 3 in cultured IFKC, contrary to cultured EKC.

Discussion and Conclusion: The expression profile of biomarkers was distinct between IFKC and EKC in situ, but less distinct when both populations were cultured in vitro. In addition, expression pattern of CD200 will be presented.

P-234

Comparative Analysis of Hair Conditions By Phototrichogram in Scalp Hair Shedding Subjects

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Objectives: Although many patients complain about their hair shedding, it is very difficult to evaluate the hair loss state objectively. The aim of the current study was to investigate the findings of phototrichogram (PT) of affected areas in hair loss with various kinds of hair shedding and to compare them with those of healthy subjects.

Approach: On a subjective global and clinical basis, 212 hair shedding subjects were classified in 88 male pattern androgenic alopecia (MPAA), 75 female pattern hair loss (FPHL), 49 chronic telogen effluvium (CTE). To compare the state of normal conditions, twenty controls not having hair loss and any other systemic disease were included. Eleven scalp sites such as right, center, left of frontal, vertical, occipital areas and right, left temporal areas were evaluated with phototrichogram.

Results: Base phototrichogram profiles of hair shedding subjects showed significantly decreased hair density and thickness than controls, respectively. Among hair shedding groups, CTE showed most low hair density. In MPAA group, hair thickness had a significant decreased in frontal and vertical areas compared to other groups and occipital areas. Also FPHL showed similar changes of hair density and thickness but the quantity of that was not great as MPAA.

Conclusion: We concluded that phototrichogram is very useful instrument to clarify the hair shedding state. Especially when subjects complain of non specific shedding without obvious hair loss, basic phototrichogram data is very informative to differentiate the exact hair loss state.

P-235

Trichogram and Immuno-Histological Analysis of the Scalp in Psoriasis

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Objectives: Is there alopecia in psoriasis? How is the inflammatory infiltrate of the scalp composed comparing uninvolved and lesional skin?

Approach: 10 patients with psoriasis were examined. Trichograms were evaluated with regard to telogen and anagen hair and compared with 20 healthy individuals. Biopsies from uninvolved and lesional scalp psoriasis were examined. Epidermal thickness was determined microscopically. In cryostat sections immunocompetent cells in epidermis and dermis were stained with monoclonal antibodies (mAb) against CD3, CD4, CD8, CD45RO, 4KB5, HLA-DR, CD1a, CD11a, CD54, CD25.

Results: With regard to trichograms, no difference in the number of anagen and telogen hairs could be found between healthy subjects, uninvolved and lesional psoriatic skin lesional hair shafts tended to be thinner and more brittle. Alopecia could not be established. Comparing epidermal thickness, lesional epidermis was about 3 times thicker than that from uninvolved sites. In the epidermal infiltrate, CD4/CD8 Ratio in Lesional Psoriatic Scalp was 0.4.

Conclusion: Hair growth is largely unaffected in psoriasis. The infiltrate of the psoriatic scalp lesions is more intense in the dermis. The findings suggest an immunological stimulation utilizing predominantly CD8+ cells in psoriatic scalp.

P-236

Comparing Phototrichoscopy – an Imaging Device – to Scalp Histopathology for the Diagnosis of Female Pattern Hair Loss

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Objectives: Evaluation of the effectiveness of phototrichoscopy for the diagnosis of female pattern hair loss (FPHL) compared to scalp histopathology.

Approach: It’s difficult to determinate if lack of hair volume and density is consequent to an initial FPHL or connected to other problems, such as chronic or acute telogen effluvium causes. In many cases scalp biopsy is a necessary step in establishing diagnosis. Since an invasive exam
is not always tolerated for patients, we studied the use of phototrichoscopy – which is faster, cheaper and non-traumatic – as a substitute to scalp histopathology since 2000 with an excellent accuracy.

Before taking the scalp biopsy, we performed a phototrichoscopy – using an adapted dermatoscope attached to the frontal lens of a digital camera at the same place that would be further compared to the biopsy.

Results: For patients with initial FPHL, phototrichoscopy and scalp histopathology were both able to show hair miniaturization.

In general, a pure CTE shows the same pattern in the frontal and occipital areas, presenting a normal biopsy and phototrichoscopy, while FPHL presents progressive miniaturization, usually severe in the frontal area and decreasing in severity towards the occipital area. Both methods presented good accuracy and correlation for presence of miniaturized follicles.

The diagnosis criteria adopted for FPHL histopathology has a problem: the “empty” follicles are not counted and so the anagen/telogen rate is affected, limiting the diagnosis criteria; with phototrichoscopy it is possible to virtually scan the entire scalp, avoiding this deficiency.

Conclusion: Compared to scalp histopathology, phototrichoscopy as a method of evaluation of FPHL is useful for diagnosis in initial stages and allows the differentiation between FPHL and CTE. As an advantage to scalp histopathology, the phototrichoscopy avoids “false negative” results and is useful for advanced FPHL with “empty” follicles.

P-237
Hair Loss Diagnosis Tool or ALGORITHM
Zemite, Inga; Riga Stradins University, Faculty of Medicine, Riga, Latvia

While there are number of reasons of hair loss, the proposed idea is that etiology depends on range of symptoms that can give clear understanding about the way what patient is suffering from and how s/he has to be investigated and treated accordingly.

Primary purpose of this job was to collect knowledge accumulated in the 10 years of trichological practice and made them useful broadly and easily in use of patient themselves and in dermatology or general practitioners practices as well.

Approach was to develop easily usable PPT file with more as 300 slides that include (1) general information about hair loss, (2) methods that can be used for diagnostic purposes, (3) questionnaire that allows getting to diagnosis of hair loss type or problem of skin, (4) short explanations that are given for every type of hair loss in conformity with current understanding of the problem. There are a lot of pictures included in the tool. For easy traveling through the tool a huge amount of hyperlinks are inserted, that gives possibility to find necessary information fast.

There is separate section for hair loss for children and teens, and additional section with drawings for hair shaft problems.

Constant research to find new ways to increase understanding about hair diseases and help trichological patients is a challenge. This algorithm is developed to make exact hair loss diagnosis, so physicians and patients are offered reliable product that performs as claimed.

Further management of specific trichological disease after diagnostic is in hand of physician.

P-238
Examination of the in Vitro Follicle Closing Technique Using Caffeine, an OECD Test Substance For Skin Absorption Tests
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Objectives: Recent investigations presented during the workshop “Follicular Penetration and Targeting” at the 4th International Meeting of Hair Research Societies in Berlin, 2004 confirmed preliminary indications that the hair follicles also play an important role in skin penetration. This study presents the first in vitro data on the follicular closing technique using static Franz diffusion cells. The feasibility of implementing a technique developed in vivo after modification for in vitro experiments is shown.

Approach: The in vitro experiments were conducted in compliance with the OECD Testguideline 428 with static Franz diffusion cells. Two formulations using caffeine, the reference substance recommended in this guideline, were applied for comparison. The follicle closing technique was implemented on different areas of human full-thickness skin (female breast and abdomen).

Results: Results were obtained from control samples with open follicles as well as from test samples with closed follicles. A separation into the different skin layers provided information about the variant amount of caffeine after the 24 hour skin absorption test. All samples were analyzed with HPLC. The samples with open follicles demonstrated a more efficient penetration route for caffeine in full-thickness skin.
Conclusion: The results in this study suggest that the follicle closing technique can be implemented for in vitro skin absorption tests. In addition, the follicular as well as the intercellular penetration pathways were used by caffeine to overcome the skin barrier. New in vitro information regarding caffeine, as one of the OECD reference substances, was found.

P-239

Effects of Cyanacrylate Skin Surface Stripping on the Percutaneous Penetration of 200 nm Polystyrol Particles in Human Skin Explants

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We recently demonstrated, that the penetration depth of topically applied particles in human skin explants depends on the size of the particles. Pre-treatment with cyanacrylate skin surface stripping (CSSS) improves the penetration of microparticles >750nm into human scalp terminal hair follicles. Skin cell targeting with functional nanoparticles below this size range, however, is a newly emerging concept in hair and skin therapy, and detailed knowledge on their penetration profile is crucial for future developments in this field. The aim of this study was to investigate the effects of CSSS on the penetration profile of solid 200nm particles in human vellus hair-bearing skin. 1471 skin sections including 698 hair follicles were analyzed microscopically in skin explants from three different donors. We found a 47% increase of nanoparticle-positive hair follicles in skin samples pretreated with 2 CSSS (65% positive hair follicles in treated with 2 CSSS vs. 33.35% hair follicles in untreated skin). The overall penetration depth along the follicular duct was also increased with 18% vs. 7% of hair follicles showing penetration deep into the hair follicle infundibulum and 16% vs. 5% showing penetration further down to deeper compartments. Consistent with previous studies, we found that the penetration depth into intermediate hair follicles was deeper than in vellus hair follicles.

In summary, CSSS increased the follicular penetration of topically applied 200nm nanoparticles in human skin explants. Its relevance for clinical applications will be a key question to be addressed in future pilot studies on human volunteers.

P-240

A New Three-dimensional Hair Follicle Model To Investigate Epidermal-Mesenchymal Interactions In-Vitro

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The human hair follicle is a highly specialized skin appendage continuously renewing itself to produce the visible hair shaft. However, the complex biological mechanisms controlling hair growth, fibre formation and pigmentation as well as the reasons for biological alterations such as hair loss or loss of pigmentation are still poorly understood. In order to evaluate principles of epidermal-mesenchymal interactions it is essential to provide a test system as close to nature as possible. The cell-cell communication of various, highly specialized cell types in this complex microcosm as well as the spatial cellular orientation determines the system’s response to endocrinal and environmental stimuli. Therefore we established a novel three-dimensional in vitro model, which allows different cell types to interact concordantly to the in-vivo situation. In this hair follicle model we integrated reconstructed dermal papillae into a pseudo dermis with dermal fibroblasts, covered by a layer of ORS keratinocytes. To show the high similarity between the reconstructed model and native human hair follicles we demonstrate the expression of characteristic follicular markers such as versican and the effect of a well-recognized potent stimulator of human hair growth in vivo, Cyclosporin A.

With this test system we set up a powerful tool to study hair follicle metabolism and epidermal-mesenchymal interactions. In combination with advanced molecular techniques it enables a deeper insight into the molecular events within the hair follicle and discloses new possibilities to elucidate the mechanisms controlling the “living” part of the hair.

P-241

Robotic Expansion of Cells for Use in Tissue Engineering of Hair

Kemp, Paul D.; Intercytex, Manchester, UK

In order for hair multiplication by follicular cell implantation to be a practical proposition, the number of cells returned to the patient must be significantly greater than the number harvested. Moreover, for this process to be commercially successful and cover the high costs of cell culture, a manufacturing facility must have the capacity to expand
the cells of many patients simultaneously. For safety reasons, each culture must be maintained in total isolation from the others to ensure that no cross-contamination can occur between the various patients’ cells. This has been a long-standing issue with organisations involved in tissue engineering, especially those involved in autologous therapies. Three basic approaches have been employed to deal with the issues, and all three will be presented and their advantages and disadvantages discussed. Intercytex is following a strategy based on a robotic cell culture system that was previously developed and proven in the area of high throughput screening. This system uses standard tissue culture vessels and dispensers to feed and passage mammalian cells although they are not yet approved by regulators for use in tissue engineering. The adoption of a robotic system will impact research decisions made early in the development of a cell culture process so that the process will be made “robot friendly”. The system will be presented and the route to regulatory approval discussed.

P-242
Large Scale Manufacturing of Cell Therapy For Hair Regeneration
Kemp, Paul D.; Intercytex, Manchester, UK

The implantation of follicular cells, expanded in culture, to induce potentially unlimited amounts of hair has been suggested ever since the work of Oliver and Jahoda. Companies are currently in Phase I and II studies to test preparations of cells for hair induction. It seems from the long history of research in this area that processes will soon be developed to successfully achieve this long sought after treatment. But how will these processes be translated into an industry whereby thousands or hundreds of thousands of people could be treated annually? Tissue Engineering is an industry now in its third decade and over a quarter of a million people have been treated by a variety of cultured cell constructs. The early pioneers developed processes often by trial and error, and only now is “best practise” beginning to emerge. The lessons learned in the past are crucial to the current development of tissue engineered hair and are the subject of this presentation. All tissue engineering processes can be broken down into six stages: “Tissue Procurement”; “Cell Isolation”; “Cell Expansion”; “Assembly”; “Shipping”; and “Application”. Regulations, industry standards, and developments in other fields are rapidly evolving and influencing each of these stages. The talk will cover these six stages and describe how an industrial process could be developed to meet the commercial needs of large scale follicular cell implantation.

P-243
Proto-Hair Development In Vitro
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Objectives: To develop an in vitro system in which to grow partially formed hair follicles that, when implanted, will continue to develop into fully formed hairs.

Approach: Hair follicle cells were prepared by enzymatic digestion followed by two sequential sievings. Cell aggregates were made and cultured to allow proto-hair development in vitro. Both freshly made and cultured aggregates were implanted onto an immune deficient mouse ear skin to test for mature hair development.

Results: Aggregates made with freshly isolated cells consistently developed hairs after implantation. Aggregates cultured in vitro consistently developed proto-hairs, which are partially formed hair follicles with distinctive morphology. Such in vitro-produced proto-hairs further developed into mature hairs after implantation. Most implants developed hairs that grew out of the skin, while some developed underneath the skin surface but failed to emerge. Histological studies confirmed immature hair structures formed from cell aggregates during cultivation in vitro.

Conclusion:
• We demonstrated that proto-hairs can be produced in vitro from cultured cells.
• We proposed a concept of the immature ‘proto-hair’ as an option for clinical transplantation.

P-244
A Comparison of Cutaneous Fibroblast Subtypes Reveals That Human Dermal Sheath Fibroblasts Exhibit Preferential Wound-Healing Characteristics, and These Are Modulated By POMC Peptides and CRH
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Although human skin contains several distinct fibroblast subpopulations, clinical evidence indicates improved wound healing in haired body sites – reflecting, possibly, the proportionately greater numbers of dermal sheath (DS) and follicular papilla (DP) fibroblasts in such skin. This in vitro study investigated fully-matched DS, DP and dermal fibroblasts (DF) isolated from five healthy individuals for proliferative, migratory, collagen-producing and contractile
Hair greying invariably manifests as part of ageing and is considered irreversible. Pigment loss in canities is due to a reduction or absence of actively melanogenic hair follicle melanocytes in grey and white hairs respectively. There is also dysfunctional pigment transfer to cortical keratinocytes as a result of melanocyte degeneration. Amelanotic melanocytes of the mid-to-lower outer root sheath (including the bulge region) are still present in white hairs but incomplete melanocyte stem cell maintenance leads to greying. Repigmentation of grey or white hairs can occur with certain medical conditions, usually as a post-inflammatory phenomenon, and as a side effect of some medications. However, most cases of repigmentation are unsustained.

We describe pronounced and long-term repigmentation of white donor hairs in a 57 year old woman who underwent hair transplant surgery for an area of frontal scarring alopecia secondary to a burn from childhood. The repigmented hairs have remained stable at more than two years post transplant. Although repigmentation of hairs is thought to be a post-inflammatory phenomenon or secondary to altered signally in the hair bulb pigmentary apparatus after surgery, the mechanisms behind the long-term upkeep of the repigmentation remain elusive. Further studies focussing on the pathophysiology of the repigmentation mechanisms will advance the understanding of the greying process. Such studies may also provide insights into strategies to reverse hair greying in the future.


P-246

Donor Site Dominance in Action: Transplanted Hairs Retain Their Original Hair Pigmentation Long-Term

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The concept of ‘donor dominance’ in hair transplantation refers to autografts which continue to maintain their integrity and characteristics after transplantation to a new site. Such hairs may retain their original texture and rate of growth. Hair transplantation for patients with androgenetic alopecia rely on this concept of donor dominance for a successful and long-lasting result. Recently, the concept of ‘recipient dominance’ in hair transplantation has been debated. In a study of patterns after hair transplantation to the scalp and eyebrows in patients affected by madarosis, Lee et al found that the greying rate of hairs approximated the recipient site rather than the donor site.

We report on the long-term maintenance of follicular pigmentation in transplanted hairs. We describe two patients affected by both androgenetic alopecia and hair greying in the transplant recipient area. They were given autografts of normally pigmented hair follicles harvested from the occipital area. More than one year post-transplantation, their donor hairs have remained pigmented long-term, despite being implanted in scalp affected by greying. In one patient the pigmented hairs have remained stable for 10 years. As the process of greying usually affects the temporal scalp first, then progresses onto the vertex and occiput later, the maintenance of long-term follicular pigmentation in our patients may be attributable to donor dominance.

P-245

Long-Term Hair Repigmentation Following a Hair Transplant For Frontal Scarring Alopecia

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Hair greying invariably manifests as part of ageing and is considered irreversible. Pigment loss in canities is due to a reduction or absence of actively melanogenic hair follicle melanocytes in grey and white hairs respectively. There is also dysfunctional pigment transfer to cortical keratinocytes as a result of melanocyte degeneration. Amelanotic melanocytes of the mid-to-lower outer root sheath (including the bulge region) are still present in white hairs but incomplete melanocyte stem cell maintenance leads to greying. Repigmentation of grey or white hairs can occur with certain medical conditions, usually as a post-inflammatory phenomenon, and as a side effect of some medications. However, most cases of repigmentation are unsustained.

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P-247
Staged Hair Transplantation in Cicatricial Alopecia After CO2 Laser-Assisted Scar Tissue Remodeling

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Objective: To evaluate whether the laser-assisted dermal remodeling before follicular unit transplantation in cicatricial alopecia (CA) could improve the vascularity of the recipient bed and to enhance the hair growth of transplanted follicles.

Approach: A high-energy ultrapulse CO2 laser with a 400-mJ pulse set at 100 Hz was used to create round serial 1.0 mm-sized holes about 3-5 mm deep into the skin with a pattern density spacing of 5 mm hole-to-hole distance. Clinical relevance was assessed over 1-month intervals. Retreatment was performed if the sclerotic tissue still remained firm and inelastic and if additional treatment was indicated by clinical examination.

Results: All patients demonstrated fibrotic scar tissue improvement and the production of a pinkish appearance after laser-treatment of 1 or 2 sessions. None of the CO2 laser-treated sites had visible new scarring or showed hypo- or hyper-pigmentation, persistent edema, or infection. In all patients, we achieved acceptable cosmetic results after only one to two FU transplant sessions at a density of 50 to 70 hair follicles/cm². Moreover, the grafted hair follicles grew hairs fast comparable to those transplanted in normal recipient areas and well covered the formerly alopecic areas.

Conclusion: Staged hair transplantation after CO2 laser-assisted scar tissue remodeling could be a useful means of achieving cosmetic improvement in cases of CA, and was found to be suitable for Asians who would be more vulnerable to stretch-back scar formation after reduction surgery.

P-248
Hair Restoration Surgery in a Patient with Stabilized Cicatricial Alopecia

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Objectives: We report a case of successful treatment of burnt out cicatricial alopecia with hair restoration surgery.

A 51 year old female patient was referred to our clinic for the evaluation of hair loss in 2001. She first noticed patches of hair loss in 1995. Histopathology confirmed our clinical diagnosis of cicatricial alopecia and was consistent with Pseudopelade of Brocq. We treated the patient with topical Clobetasol twice daily for 2 years. We stopped the topical treatment in 2004. The lesions remained stable.

Approach: Hair restoration surgery was performed in 2005. A total of 1200 follicular units were transplanted into the scarred areas.

Results: The patient showed good hair density and scalp coverage 6 months after the surgery. She discovered a great improvement in her quality of life.

Conclusion: Hair restoration surgery is a treatment option for burnt out cicatricial alopecia. The lesions should be stable without treatment for at least 1 year. The potential of reactivation of the cicatricial hair loss post surgery has to be discussed with the patient in detail.

P-249
Cyclic Dislocation of Hair Follicular Stem Cells and Its Importance in Hair Transplantation

Tamazashvili, Tamaz;1 Burnadze, Koba;2 Mchedlishvili, Maya;2 Bebiaishvili, Irakli;3 Chuchulashvili, Katie;3

Background and Objectives: Apoptosis is a major biological process in the hair follicle cycling and is controlled by pro- and anti-apoptotic proteins. Because stem cells in general need to be protected from apoptosis, the presence of the apoptosis-suppressing Bcl-2 protein may be used as an indicator of the stem cells population in the hair follicle. Localization of stem cells at the bulge region of the hair follicle has been proven by some recent research. Stem cells have been detected in the area of attachment of arrector pili muscle to the follicle. However, until now there is scanty information about dislocation of stem cells during cyclic development of hair follicles.
Methods: Hair follicles were derived from the occipital area of the patients who underwent the hair transplantation procedure. For immuno-histochemical studies Bcl-2 antibodies (clone 124) were used.

Results: The data obtained clearly indicated that stem cells are not only positioned in the area of attachment of arrector pili muscle, but can surround the hair follicles and spread towards distal and proximal parts of the follicle. This phenomenon is especially obvious during the initial stage of anagen, when migration of the stem cells has been noticed along with the downward development of the follicle. Mono-positional localization of stem cells in the bulge area was more characteristic for the telogen phase. To prevent the possibility of the removal of mono-positional stem cells, a special method of hair follicular dissection has been developed. The essence of this method is to avoid inter-follicular dissections at the areas of attachment of arrector pili muscle.

Conclusion: Thus, new information has been obtained about cyclic dislocation of follicular stem cells that allows maximum preservation of those cells during follicular dissection and improves the results of hair transplantation.

P-250
Use of Phototrichoscopy in Female Pattern Hair Loss to Evaluate Donor and Recipient Areas for a Hair Restoration Surgery

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Objectives: Evaluate the use of phototrichoscopy for donor and recipient areas in female pattern hair loss.

Approach: Clinical treatment for FPHL can’t reverse severe miniaturization and hair restoration surgery (HRS) is a valuable option. The success of this procedure is related to a correct donor area evaluation. We perform phototrichoscopy since 2000: the best candidates for HRS has localized frontal miniaturization and preserved donor area, while those with diffuse thinning and compromised donor area are not.

Results: The phototrichoscope is a device similar to a dermatoscope attached to a digital camera (Sony W1). After parting the hair a clear alcoholic gel is applied over the contact glass and pressed over the scalp. We document four areas: central frontal area, usually the most compromised (1) fronto-parietal area, moderately affected (2), the lateral occipital area, could be mildly compromised (3) and the central occipital area, usually the fuller area (4). In all areas we evaluate the density and miniaturization.

• Good candidate:
  Miniaturization – intense in the central-frontal area, moderate in the fronto-parietal area and absent in the occipital area. Density – severe reduction in the central-frontal area, mild reduction in the fronto-parietal area and preserved in the occipital area.

• Not indicated:
  Miniaturization – severe in the central-frontal area, intense in the fronto-parietal area and also moderate to intense in the occipital area. Density – moderate reduction in the central-frontal area and fronto-parietal area. Moderate reduction in the occipital area.

Conclusion: Good candidates have a great contrast between donor and recipient areas to justify a hair restoration procedure. Phototrichoscopy allows the dermatologists to correct evaluate the entire donor area in reference to miniaturization and density besides helps the surgeon finding a “safe donor area”.

P-251
Case Report: Lipedematous Alopecia – a Surgical Outcome

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We report the first case of surgical correction of lipedematous alopecia. Lipedematous scalp is characterized by asymptomatic boggy swelling of the scalp. It has been reported mainly in African-American women. When hair loss is present, this condition is termed lipedematous alopecia. The etiology and natural course are unknown. No effective treatment has been described. Our patient is a 67-year-old woman who presented with a 10-year history of a gradually enlarging boggy swelling with overlying alopecia over the vertex of her scalp that measured 61mm x 35mm. Ultrasound showed thickening of the underlying subcutaneous tissue in this region. Biopsy revealed hyperplasia of subcutaneous tissue and loss of hair follicles. Surgical excision of the alopecia and surrounding swelling was performed and primary closure was achieved with minimal skin tension. There is no evidence of relapse at three months post-operation.

References:
P-252
A Relationship Between Early Greying and Extent of Male Pattern Baldness?

Davies, Paul G.; Randall, Val A.; University of Bradford, Bradford, UK

Both androgenetic alopecia and loss of hair pigmentation advance gradually with increased age. However, androgenetic alopecia is a predominantly male, androgen-dependent condition, while androgen-independent canites occurs in both sexes. Observations suggest that men with early pigment loss retain scalp hair, but this may be an inaccurate impression due to the greater visibility of white hair.

To determine whether there is any relationship between these processes, the extent of balding and pigment loss was compared between men over 18 who were, or were not, greying before 30. A single observer scored balding extent using the Hamilton scale and pigmentation loss on a scale of 1 to 5 in 364 Thais. A further 216 Europeans completed the same questionnaire assessing their own hair status. Data was analysed in each group separately and combined.

Mean ages at assessment did not differ between men grey before, or after, 30: 26 early Thais 38.7 years, others 39.4; 50 early Europeans 49.4, others 52.8. The extent of both male pattern balding and pigment loss was positively correlated with increasing age whenever canites started. Aging was more strongly correlated with pigment loss.

Mean pigment scores were higher with pigment loss before 30; contrastingly, mean Hamilton scores were lower.

These results concur with the age-related nature of both conditions and indicate that early pigment loss remains advanced with age. However, the lower Hamilton scores in early canites opposes predictions if both were solely aging related. Early pigment loss appears inversely related to male pattern alopecia extent.

P-253
Expression of Human Cultured Epidermal Melanocytes in the Reconstructed Mice Hair

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To elucidate the behavior of melanocytes (MCs) in the hair follicles, we have constructed reconstructed mice hair follicles that included xenografted human MCs. These MCs could functionate in the graftings of epidermal and dermal cells derived from albino mice and turned the color of white hairs into gray color. Melanin pigments were observed in the reconstructed epidermis and hair follicles and human MCs were observed around dermal papilla. The increased amounts and tyrosinase activities of MCs increased the darkness of reconstructed hair colors. By ACK2 treatment of pregnant ICR mice, the color of reconstructed hairs increased significantly. For the further studies, we used Wsh/sh mice as donor mice, which lacks both follicular or epidermal melanocytes in their skin. The color of reconstructed hairs also becomes dark significantly. These results strongly suggested that human MCs can functionate effectively in the vacant niches in the ACK2 treated mice and Wsh/sh mice that lacked their endogenous melanocytes. After plucking out all reconstructed hairs, secondary hairs were re-grown in the same area and their colors became lighter compared with the color of the first reconstructed hairs. This result suggested that xenografted melanocytes could not maintained well but partially functionate in the next hair cycles.

P-254
The Reflectance Confocal Microscopy in the Study of Hair Follicle Pigmentary Unit

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The biology of hair bulb shows how close is the hair growth cycle and follicle melanogenesis, and how many different factors contribute to hair aging and canities. Comparing the response of normal hair follicle pigmentary unit during catagen and telogen phase, and the effects of some immunological diseases on the hair bulb and melanogenesis, it is possible to have evidence about the role of apoptosis in premature aging of hair follicle melanocytes. The evidence that aging and pathological stress produce the alteration of the “unit” anagen phase-melanogenesis and therefore the reduction of follicle melanocyte function and decrease in hair production, also provides the evidence that only acting on both the follicle compartments it could be possible to reduce hair graying.

The Reflectance Confocal Microscopy (RCM) is a useful method to study the changes of hair follicle pigmentary unit in vivo in normal, aging and pathological hair, and to evaluate the effects of some active principles on anagen prolongation and melanocyte function. We tested the main expressions of apoptosis in catagen graying hair, to compare the findings from RCM, and to show if some treatment had any effect to slow graying hair.

We used RCM in a clinical evaluation on 60 subjects with graying hair trying to clarify the different pathways of graying, and the effects of topical liposome containing specific active principles on follicle pigmentary unit. At the end of the clinical trial we can conclude that RCM is
an useful device to evaluate the hair follicle pigmentary unit in vivo, and that it is possible to evaluate the efficacy of active principles (5 different actives, used in liposome preparation), showing that 3 of these gave a reduction of graying hair (p < 0.01) in 65% of subjects treated with active principles, respect no difference in placebo group.

P-255

Breakthroughs in the Characterisation, Location and Tracking of Modifications in Fibrous Proteins – Unravelling the Mystery of Wool Photodegradation

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UV-induced photomodification of proteins has been implicated in a diverse range of deleterious processes including hair damage and skin ageing. In the case of wool, exposure to direct sunlight results in gradual photoyellowing of the fibres, while exposure to sunlight filtered through glass results in photobleaching. The underlying chromophoric species and processes responsible for this degradation have remained a mystery through over forty years of research.

For the first time, yellow chromophore-containing photo-oxidation products have been directly characterised within the proteins of photoyellowed wool fabric utilising ESI-MS/MS. To date, fifteen chromophores have been identified and located within known wool peptide sequences. Eleven tryptophan-derived photo-modifications were characterised, including hydroxytryptophan, kynurenine derivatives, tryptophan diones, carboline derivatives and nitrotryptophan. Four tyrosine-derived modifications have been characterised, namely dopa, topa, dityrosine, and a previously unreported modification consistent with a hydroxylated dityrosine residue. These modified residues were located within a range of wool peptides, but particularly in peptides derived from wool intermediate filament proteins. The range of photo-oxidation products characterised provides direct insight into photochemical pathways leading to protein photodegradation, while supplying experimental evidence in support of current theories as to ROS-derived photomodification mechanisms. These breakthroughs have been extended to the direct tracking of specific marker photomodifications through a range of irradiation and treatment protocols.

P-256

Age-Associated Down-Regulation of Catalase in Human Scalp Hair Follicle Melanocytes

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Although epidermal melanocyte numbers gradually decrease with age, hair graying (canities) is often dramatic, suggesting a different “melanogenetic clock” for these two cutaneous melanocyte subtypes. Currently, canities is thought to result from a combination of reactive oxygen species-associated damage in hair follicle melanocytes (HFM), impaired anti-oxidant status and failure of melanocyte stem cell renewal.

To explore the role of oxidative stress in canities we examined catalase expression in situ and its activity in cultured HFM derived from young and aged donors. Cell survival, catalase expression / activity, and p38 stress kinase expression were examined after exposure to exogenous H2O2.

Results showed that catalase expression in situ was reduced in HFM with advancing age and contrasted with the high expression of this anti-oxidant enzyme in hair follicles from young donors. Catalase expression and activity in vitro was also higher in young donor HFM compared to aged donor cells. By contrast, no age-related change in catalase was observed in matched dermal fibroblasts. Catalase expression / activity were up-regulated after exposure to H2O2 in young, but not, aged donor HFM. This correlated with high cell survival after exposure to this oxidant in young donors. HFM from aged donors expressed higher baseline levels of p38 stress kinase compared with younger donors, and this was further increased after exposure to H2O2.

In summary, an age-dependant decrease in catalase expression / activity in HFM support the view that a reduction in anti-oxidant defence capability may contribute to loss of hair pigmentation with age.
P-257
Photo Damage of Human Hair: Color Changes as a Function of Hair Pigmentation

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It is well known that keratins are damaged by exposure to solar radiation, predominantly by ultraviolet wavelengths. In a previous work (Journal of Photochemistry and Photobiology B: Biology, 74/2-3, 109-117, 2004) we showed that UVA is the main radiation responsible for color changes and that UVB radiation is responsible for the higher protein loss in human hair. In this work we show that the effect of long-term exposure to UV and visible radiations depends on hair pigmentation. Blended virgin blond, red and dark-brown Caucasian hairs were irradiated with a 125 W mercury vapor (full or UVB filtered radiation), in controlled temperature, humidity and radiation dosage conditions. Color data was obtained by reflectance spectrophotometry, using CIEE L (lightness coordinate), a (green-red coordinate) and b (blue-yellow coordinate) measuring system. The following trend is observed for the irradiated hair samples: all hair types become clearer after lamp exposure, quantitatively measured as an increase in L values. On the other hand, changes in the true color parameters (a and b) depend on the hair type and wavelength range. Blond hair turns yellower after full radiation exposure, but not when UVB radiation is filtered. Dark-brown and red hairs turn yellower after both full radiation exposure or UVB filtered radiation exposure. Regarding changes in redness, blond hair turns less red and dark brown turns redder after both full radiation exposure or UVB filtered radiation exposure. Red hair turns redder after full radiation exposure, but not after UVB filtered radiation exposure. Our results show that hair color damages caused by UV and visible light exposure are different for each hair type. Since we have other photosensitive components on hair, adding to the melanin chromophores, this behavior is probably related also to the overall hair chemical composition.

P-258
Expression of BMP2, FGF5 and Follistatin mRNA Throughout the Rat Hair Cycle

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A number of candidate molecules have been implicated as key regulators of the hair cycle including Follistatin (FST) and several members of the bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) families. Evidence from FGF-5 mouse knockouts has shown that it may play an important role in the switch from anagen to catagen. TGF-α superfamily members are also involved in follicle initiation and cycling in vitro, as well as interacting with growth factors such as the BMPs.

Objectives: The aim of this investigation was to determine the role, if any, of BMP-2, FGF-5 and FST in the control of the rat hair cycle.

Approach: The expression of BMP-2, FGF-5 and FST mRNA in 40 rat skin samples, taken throughout the hair cycle was quantified using real time PCR employing pre-validated TaqMan(r) assays. All data were normalised against ribosomal 18S RNA. Skin samples were histologically classified into four stages of the hair cycle; early and late anagen and early and late catagen.

Results: Analysis of the data revealed a 6-fold increase in FGF-5 mRNA levels from early to late anagen (p<0.0001). BMP-2 mRNA levels were significantly higher in late compared to early catagen (p=0.006) and FST showed a gradual increase in expression from early through to late anagen and early catagen, peaking at 1.6-fold increase during late catagen (p=0.005).

Conclusion: These results suggest an important role for FGF-5 in the transition from anagen to catagen, and perhaps indicate a concentration dependent effect of BMP-2 and FST for transition into follicle shutdown.

P-259
Requirement of Hair-Follicular Dermal Sheath for Growth of Hair Shaft

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Objectives: The rat dermal papilla (DP) cells in multiple subcultures retain the capacity to induce epidermal cells to differentiate into hair follicles when transplanted to the back skin. However, these hair follicles were not encapsulated with the dermal sheath (DS) and did not develop the hair shaft. We observed that these DP cells induced hairs (the hair follicle with the shaft), when transplanted with DS cells. In the present study, we examined the role of DS cells in the induction of hairs by DP cells.

Approach: Primary EGFP-labeled DS cells were obtained by culturing peribulbar dermal sheath cups of vibrissa follicles of EGFP-transgenic Wistar rats. DP cells from syngeneic wild-type rats were subcultured and labeled with Dil. Hair-inducing ability of each of DP and DS cells,
and of the mixture of both types of cells were examined by transplanting the test cells to the back of immunodeficient nude mice.

Results: DS cells (passage 1, p = 1) and DP cells (p = 60) each alone induced a few hairs and no hairs, respectively. However, the DP and DS cells (mixed cells) frequently induced longer hairs. Histological examinations demonstrated that the DPs of the induced hairs contained Dil-labeled cells. EGFP-positive cells were located at the peribulbar dermal sheath cup of follicles.

Conclusion: These results suggested that the DS formation in hair follicles is important for the growth of hair shafts and that DP cells cooperates with DS cells in hair induction.

P-260

Alopecia Mucinosis: Premature Hair Follicle Regression As Evidenced By Increased Catagen Phase Follicles On Horizontally Sectioned Scalp Biopsies

Miller, Jeffrey J.; Ioffreda, Michael D.; Penn State College of Medicine, Hershey, PA, USA

Two cases of primary, idiopathic alopecia mucinosis were characterized by rapidly progressive hair shedding which resulted in patchy, reversible alopecia. Besides the typical accumulation of mucin in the outer root sheath of hair follicles, the most characteristic finding on transversely sectioned scalp biopsies from our two patients was a greatly elevated catagen-telogen count, composed primarily of follicles in catagen(avg 68% of resting-phase follicles), with the remainder being telogen germinal units. The infundibular portion of follicles in catagen did not show hair shafts in the follicular canal, suggesting loss of the hair shaft prior to telogen. The fact that hairs obtained on gentle hair pull appeared to be dystrophic anagen hairs lends support for an anagen effluvium in primary, idiopathic alopecia mucinosis.

P-261

Hox-Lacz Reporter Gene Mice as Tools For Dissecting Hair Follicle-Specific Transcriptional Control Elements Regulating the Dynamic Patterns of Hox Gene Expression During Hair Follicle Morphogenesis and Cycling

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1. Medical University of South Carolina/Dept Medicine, Charleston, SC, USA; 2. The Jackson Laboratory, Bar Harbor, ME, USA

Objectives: Members of the Hox family of transcriptional regulators are known to play pivotal roles in cutaneous and follicular patterning and cycling. Understanding these functions requires to define the dynamically changing Hox expression patterns during follicle morphogenesis and cycling, and information about transcriptional regulation mechanisms regulating these complex patterns.

Methods: Starting with Hoxc13, located at the 5’ end of the Hox cluster, we proceeded in the 3’ direction to prepare various Hoxc-lacZ fusion constructs that contained the corresponding Hox promoters and surrounding regulatory sequences. Transgenic mice carrying these constructs were analyzed by X-gal staining of skin at different stages of the hair cycle. Authenticity of specific Hox-lacZ expression patterns with regard to corresponding endogenous gene expression patterns was validated by in situ hybridization analysis. Hair follicle-specific control elements were mapped by deletion analysis in vivo.

Results: This approach revealed novel patterns for several Hox genes that were difficult to discern by conventional expression analysis. For example, unlike Hoxc13-lacZ, which was globally expressed in all hair follicles, expression of both Hoxc11- and Hoxc10-lacZ was restricted to follicles in posterior regions and exhibited complex patterns, predominantly in the dermal papilla, the matrix and the cortex. The cortical patterns suggested cyclical activity in anagen follicles as we observed two separate zones of lacZ activity along the proximal-distal axis of differentiation in that compartment. Our deletion analysis indicated that these patterns are regulated by separate and autonomously acting control elements.

Conclusions: Hox-lacZ reporter gene mice are useful tools for (i) defining dynamic changes of Hox expression patterns during the hair cycle at high resolution and with great sensitivity, (ii) monitoring responses of Hox-expressing cells to specific stimuli, (iii) isolation of hair-specific cis-regulatory elements, and (iv) isolation of distinct Hox-expressing cell populations.

P-262

Atrophy of Sebaceous Glands in Two Cases of Pityriasis Amiantacea

Miller, Jeffrey J.; Ioffreda, Michael D.; Fogelberg, Anneli; Penn State College of Medicine, Hershey, PA, USA

Pityriasis amiantacea is a well-recognized, inflammatory condition manifested by thick, asbestos-like shiny scales on the scalp. We herein present two cases of pityriasis amiantacea along with newly reported histological findings. Both patients presented with asbestos-like scale of acute onset involving the scalp diffusely. Four millimeter scalp biopsies sent for horizontal sectioning revealed significant atrophy of sebaceous glands. After successful treatment with
fluocinolone acetonide oil, repeat biopsies for horizontal sectioning revealed normal sebaceous gland morphology. We will focus our discussion on the relationship of the gross findings of thickened scalp scale and the microscopic findings of sebaceous gland pathology.

P-263
Hair Cycle-Dependent Changes of Alkaline Phosphatase Activity in the Mesenchyme and Epithelium in the Hair Bulb of Mouse Vibrissal Follicles

Iida, Machiko;1 Ihara, Setsunosuke;2 Matsuzaki, Takashi;2
1. Tottori University, Tottori, Japan; 2. Shimane University, Matsue, Japan

Alkaline phosphatase (ALP) activity was detected in the bulbar mesenchyme and epithelium in mouse vibrissal follicles. Its localization and strength dramatically changed during the hair cycle. The activity in the dermal papilla (DP) was moderate in very early anagen, reached a maximal level in early anagen, decreased at the proximal region of DP after mid anagen, and was kept at a low level during catagen. The bulbar dermal sheath showed a strong ALP activity only in early anagen. Although most bulbar epithelium did not show ALP activity, germinative epidermal cells that were adjacent to the ALP-negative DP cells became ALP-positive in mid anagen and rearranged in the area surrounding the DP in mid catagen. The bulbar dermal sheath showed a strong ALP activity only in early anagen. Although most bulbar epithelium did not show ALP activity, germinative epidermal cells that were adjacent to the ALP-negative DP cells became ALP-positive in mid anagen and rearranged in the area surrounding the DP in mid catagen. During catagen, the outermost layer of bulbar epithelium became ALP-positive, which should be follicular epithelial precursors migrating from the bulge. Before the initiation of hair formation, ALP activity in the bulbar epithelium rapidly decreased and that in DP increased instead. These dynamic changes of ALP expression might be related to DP's functions in hair induction, and also to reconstruction of the hair matrix during the hair cycle.

P-264
Role of Insulin-like Growth Factor Binding Protein 5 on Human Hair Shape

Hachiya, Akira;1,2 Sriwiriyanont, Penkanok;2 Yoshida, Hiroshi;1 Ohuchi, Atsushi;1 Moriwaki, Shigeru;1 Kitahara, Takashi;1 Takema, Yoshihito;1 Boissy, Raymond E.;3,4 Visscher, Marty O.;3 Tsuboi, Ryoji;2
1. Kao Biological Science Laboratories, Haga, Tochigi, Japan; 2. Department of Dermatology, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan; 3. The Skin Sciences Institute, Division of Neonatology, Cincinnati Children's Hospital Medical Center, Cincinnati OH, USA; 4. Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH, USA

The curliness of the hair shaft is determined by the distributions of ortho- and para-cortical cells, the quality and the quantity of keratin associated proteins, the amount of disulfide bonds between the hair proteins, the activity of transglutaminase and/or other keratinization-related enzymes in hair root, and the shape of the hair follicle itself. Recently, it has been reported that forced transgenic expression of insulin-like growth factor binding protein 5 (IGFBP5) in the hair follicle of Foxn1::dnFgfr2-IIIb transgenic mice caused the reduction in the size of hair medulla and resulted in the bending hair shaft, suggesting that FGF signals specifically regulate the structure of hair shaft medulla via IGFBP5. However little is known about the involvement of IGFBP5 on human hair shape. In this study, we examined the role of IGFBP5 with isolated human kinky and straight hair follicles. Real time quantitative RT-PCR analysis suggested that expression of IGFBP5 was higher in kinky hair follicles than that in straight fair follicles. When immunohistochemical analysis was performed, IGFBP5 was asymmetrically expressed in the inner layer of outer root sheath, not in medulla, in kinky hair follicles while the localization of IGFBP5 was found to be evenly dispersed in straight hair follicles. Finally, supplemented human recombinant IGFBP5 in kinky hair follicles organ culture was shown to inhibit hair growth and to significantly augment the degree of hair shaft bending compared to control. Taken together, these findings suggest for the first time that asymmetrical IGFBP5 expression contributes to human hair shape.

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Exhibits

Booth 1 & 2

**Johnson & Johnson Healthcare Products Division of McNEIL-PPC, Inc.**

Men’s ROGAINE® 5% minoxidil foam is the first and only, easy-to-use foam approved by the FDA in the US to treat hereditary hair loss.

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Barrier [description not available]

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Booth 6

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Hair Science specializes in hair diagnosis and restoration system. FOLLISCOPE is a photothichogram system for hair evaluation. FOLLIPEN is for follicular unit hair transplantation.
HOTEL VANCOUVER

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Satellite Symposia Schedule

Wednesday, June 13th
16:30 – 18:00

Satellite Symposium

**Title:** Unwanted Hair Growth – Treatment Options and Impact on Quality of Life

**Location:** Pacific Ballroom

**Faculty:**

**Valerie D. Callender, MD** – Symposium Chair  
Clinical Assistant Professor of Dermatology, Howard University College of Medicine,  
Director, Callender Skin and Laser Center, Mitchellville, MD

**Harvey Lui, MD**  
Professor and Chairman, Department of Dermatology and Skin Science, University of British Columbia  
and Vancouver General Hospital  
Director, Lions Laser Skin Centre, Vancouver General Hospital

**Joseph D. Jackson, PhD**  
Pharmaceutical Research Institute, Bristol-Myers Squibb Co. Princeton, NJ

**Objectives:**

1. Become up to date on the treatment options for managing the complicated issue of pseudofolliculitis barbae (PFB).
2. Develop knowledge on the latest data regarding combining drug therapy with laser hair removal for unwanted facial hair (UFH).
3. Learn about the impact on quality of life when unwanted facial hair is treated and the science behind the measurements.

UFH is a common condition affecting millions of women all over the world. Many of these women are extremely bothered by their UFH and actively seek treatments hoping for successful management of the condition. The impact on patient quality of life can be significant whether the UFH is on the lip and chin or the more difficult to treat issue of PFB.

Thursday, June 14th
12:15 – 13:45

Lunch Satellite Symposium

**Topic:** Ethnic Hair

**Location:** Columbia Room
Friday, June 15th
06:30 – 07:45
Satellite Session (closed session, by invitation only)
Title: Hair Science: From Bench-top to Bottle
Location: The Boardroom

Saturday, June 16th
12:05 – 13:30
Lunch Satellite Symposium
Title: Future Of AGA Management
Location: Columbia Room
Faculty:
Wilma Bergfeld, MD
Head of Clinical Research, Department of Dermatology, Cleveland Clinic Foundation
David Cohen, MD
Associate Professor, Director of Allergic, Occupational, and Environmental Dermatology,
New York University School of Medicine
Elise Olsen, MD
Professor of Medicine, Divisions of Dermatology and Oncology, Duke University Medical Center
Rita Wanser, BS
Associate Director, Johnson & Johnson Healthcare Products, Division of McNEIL-PPC, Inc.
Ken Washenik, MD, PhD
Medical Director, Bosley, Dept. of Dermatology, New York University School of Medicine
Objectives:
Androgenetic alopecia (AGA) is by far the most common cause of hair loss. Minoxidil was the first agent shown to promote hair regrowth and continues to be a treatment of choice for AGA. This symposium will provide the attendees an overview of minoxidil, its history, and the new foam formulation. It will include discussions on the advantages of the foam vehicle and the clinical safety and efficacy of the new foam formulation. This session will also feature discussions on combination therapies with minoxidil and future treatment modalities in the management of androgenetic alopecia.