O-01-1

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

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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 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

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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

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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

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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, Bmp receptor ablation specifically in DP cells in a novel in vitro/in 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 DP's 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

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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

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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

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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 transcriptonal 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 know to the general public, but less well know 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 cm^2.

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/cm^2. 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.

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 day43 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-Differrentiation of the Foot Pad Epidermis Towards Folliculogenesis

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Objectives: During skin development, epithelium and mesenchyme interact each other and give raise 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 [SWGs]) 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 centres 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. LYVE-1 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.

O-7A-5

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 cm^2 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.

O-7B-1

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.

O-7B-2

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

Affymetrix array (GeneChip Mouse Genome 430 2.0) and immunohistochemistry (IHC). Microarray analysis revealed that expression of most enzymes and proteins involved in retinoic acid (RA) synthesis were increased, while expression of RA degradation enzymes and binding proteins were decreased in AA compared to sham controls at 10, 15, and sometimes 20 weeks after grafting. Histological analysis of these samples found that 10, 15, and 20 weeks after grafting AA hair follicles were primarily in late anagen/early catagen, while their control hair follicles were primarily in telogen. To determine if differences in mRNA expression were due to the disease or the hair cycle immunohistochemistry was performed with antibodies against three proteins involved in RA synthesis that were increased in AA as determined by the microarray. This analysis found that all three proteins were increased in the companion layer of dystrophic follicles from AA mice. Normal looking follicles from AA mice and their sham controls had similar expression patterns to normal wax stripped mice of the same stage. These data suggest that increased RA synthetic proteins detected by microarray are partly due to both hair cycle differences and follicular effects of AA.

O-7B-3

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 £100 on wigs and hairpieces with 16 having spent over £1,000. 59 (90%) reported having visited one or more alternative practitioners for their alopecia. 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 C57/BL/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 Proopiomelanocorpin (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 ($p's<0.05$), 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 hyperaesthetic 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.

Results: fifty-five subjects were enrolled (34:21, Caucasian: Asian). Mean age of onset was 34.2 years. The majority (89%) experienced gradual progression and increased shedding (62%). Family history was positive in all. Onset in perimenopause, puberty, or postpartum occurred in11%, 4%, and 4%, respectively. Patterns observed: 1) central thinning (96%) with lateral and/or vertex involvement in all (lateral, 92%; vertex, 75%; both, 68%); 2) lateral hair loss with bitemporal recession and frontal hairline breach (2%); and 3) global thinning (2%). Associated bitemporal recession was seen in 36%, frontal accentuation, 22%, peripheral occipital thinning, 15%, and frontal hairline breach, 13%. Of those in whom hair transplantation was an option, 90% had an adequate to excellent donor site. TSH was abnormal in 6%; ferritin < 40 _g/L in 50%; no subjects were diagnosed with hyperandrogenism.

Conclusions: The current models for FPHL do not fully capture the range of patterns observed: lateral thinning is an associated feature in most; peripheral occipital thinning, known to occur in men, is not uncommon in women; and global thinning can be seen. Most females with moderate patterned thinning are candidates for hair transplantation. A large-scale study is needed to further explore these novel findings.

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 required for anagen onset (Wnt-b-catenin, TGF-a, TGF-b, Stat-3, Stat-1), epithelial signal to dermal papilla (PPARd, IGF-1), hair shaft differentiation (Notch, Msx2, KRTs, KAPs), and anagen maintenance (Msx2, Activin, IGF-1) were downregulated; and genes for catagen (BDNF, BMP2, BMP7, VDR, IL1, ER) and telogen induction and maintenance (VDR, RAR) were upregulated. In contrast, the transcriptional profile of SA was comparable to other aging systems. In SA, 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 HpaII. 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 reversibly. 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 (DHRS9), 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^{abJ}, Scd1^{ab2J}), 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^{ab2J} 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.

Results: The strongest associations with AA overall were with the AIRE 7215C variant ($p=2.7x10^{-10}$). We now report a strong association between the AIRE 7215C variant and failure of DPCP therapy such that the success rate in DPCP treated patients negative for the AIRE 7215C was 30% but this fell to 10% in patients bearing the disease allele ($p<0.0001$).

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 familiy 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 ($Foxn1^{nu}$) 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 cells 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 lpid 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 calmegin 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 absorbtion in melanin is lower,will be better tolerated in Asians.

Thus epidermal damage or hypo pigmentation is lower About IPL (intense pulsed light):

It is much more suitable for blonde or grey hair .It has different wavelengths and less risk of scar and side effects as observed in our patients too.

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 a autocrine manner, recapitulating the reciprocal pathomechanism between androgen and TGF-b1.

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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. Immuneoreactivity 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 thyroglubulin 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.