

## Posters

### **P1: Four Cases of Acquired Localized Hypertrichosis Associated with Trauma**

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**Key words:** Acquired localized hypertrichosis, trauma

Hypertrichosis is the increase of hair density or length beyond the accepted normal range for particular age, race, or sex. Because hypertrichosis is the excessive growth of hair on androgen-independent areas, it differs from hirsutism, which refers to hair growth in women on androgen-dependent areas of the body. It is usually classified based on the time of onset (congenital vs. acquired) and distribution (generalized vs. localized), and may either be an isolated finding or be associated with other abnormalities. A great variety of stimuli have been noted to cause local increase in hair growth. Especially, external stimuli such as infective or inflammatory dermatoses, drugs and trauma can produce localized hypertrichosis. Although the pathogenesis of localized hypertrichosis is not well understood, localized arterial hyperemia, increase of growth factors and cytokines related to hair growth, and angiogenesis after external stimuli have been proposed as the possible hypotheses. We report our experiences of acquired localized hypertrichosis occurred on lower legs after physical trauma in four women.

### **P2: The Combination Therapy of Cyclosporine and Methylprednisolone on Severe Alopecia Areata: 3-year Follow-up Study**

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**Key words:** Combination therapy, alopecia areata

Although completely effective treatment of severe alopecia areata (AA) has not been established, several study showed that the combination therapy of cyclosporine and methylprednisolone may be a useful treatment for severe AA. But, relapses are also common upon withdrawal of the medication. This study was designed to evaluate the recurrence rate after discontinuation and long term side effect of the combination therapy of cyclosporine and methylprednisolone on severe alopecia areata. A total of

25 patients with severe AA (2 in ophiasis type, 6 in alopecia totalis, 17 in plurifocal type) were treated with a combination of cyclosporine (100 mg~200 mg daily) and methylprednisolone (10 mg~30 mg daily), and then the dose of methylprednisolone were tapered within 2 months and the dose of cyclosporine were decreased gradually for 6 months. 3 year later after the cessation of medication, we follow up the 25 patients and asked the status of hair loss of the patients and long-term side effects. 20 (80%) of 25 patients (2 of 2 in ophiasis type, 6 of 6 in alopecia totalis, 12 of 17 plurifocal type) have experienced the recurrence of alopecia. Although some patients suffered from GI trouble, hypertrichosis during taking medication, Long-term side effects of the medication were not observed in all patients. This study shows that although combination therapy with cyclosporine and methylprednisolone may be a useful treatment for some patients with severe AA and side effect was not significant, the recurrence rate after discontinuation was very high.

### **P3: A Study Upon Parameters Useful for Evaluating the Antidandruff Efficacy of 1% Zinc Pyrithione Shampoo**

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**Key words:** Zinc pyrithione, dandruff

Dandruff is a chronic scalp or face condition developed by exfoliation of stratum corneum. It waxes and wanes in accordance with the changes of climate, environment, stress and medication. There are so many drugs, shampoos, and cleansers used to control dandruff. However, their efficacy is not confirmed by objective parameters or evaluation methods in most cases, which makes consumers confused. Therefore, a randomized, double blind, placebo-controlled study was performed to investigate the antidandruff efficacy of shampoo containing 1% zinc pyrithione. Thirty male patients aged between 20 to 50 years without skin diseases other than dandruffs were divided into treatment and control groups. They were told to use shampoo for 4 weeks. Parameters were assessed including subjective satisfaction score, objective satisfaction score, scalp surface evaluation by folliscope, hydration by corneometer, sebum secretion by

sebumeter and erythema index by chromameter. In the treatment group, parameters such as subjective satisfaction score, objective satisfaction score, scalp surface and sebum secretion were improved whereas hydration or erythema indexes failed to show any significant difference. In the present study, we confirmed the antidandruff efficacy of 1% zinc pyrithione shampoo, and parameters mentioned above can be used as objective indicators for evaluating antidandruff shampoo in the future.

#### **P4: PLA-particles as a Drug Delivery System for the Treatment of Follicular and Sebaceous Gland Disorders**

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**Key words:** Drug delivery systems, PLA-particles, controlled drug release, hair disorders, sebaceous gland disorders

The need for a biodegradable DDS for the treatment of hair follicle associated diseases set the focus on PLA-Particles since increased and selective penetration of such particles in the human hair follicle were observed in earlier investigations. In this study we investigated the release properties of a lipophilic dye incorporated in PLA-particles in excised human skin with the use of fluorescence microscopy. After particle accumulation in the infundibulum of human vellus hair follicles the dye was partially released and stained not only the follicle but also the sebaceous gland. The latter remained stained for more than 24h indicating a prolonged release and a partially selective targeting. The use of a biphasic hydrophilic/lipophilic suspension enabled the *in vitro* simulation of the interaction of the PLA-particles suspension with the sebum in the hair follicle. A dye release was detected and quantified by means of fluorescence spectrophotometry. Microscopic analysis of the nanoparticles revealed a structural change and the formation of conglomerates at the interface between water and organic solvent. These results suggest PLA-NP to be potential candidates for an optimal DDS for the treatment of follicular and sebaceous gland disorders. The prolonged release of the incorporated drug would enable to achieve constant drug levels within the hair follicle, which is useful for the treatment of hair related diseases such as alopecia or hypertrichosis. Furthermore, targeting of the sebaceous gland is of utmost importance for the treatment of sebaceous gland related disorders such as acne and rosacea.

#### **P5: Comparison of Hair Shaft Repairing Efficacy Between Panthenol and Two Molecular Weights of Hydrolyzed Protein**

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The diverse causes of extrinsic hair shaft damage have been documented, and roughly it can be divided into physical causes and chemical causes. Among them, permanent waving and hair dyeing are very important causes in a cosmetic point of view. Recently, different patterns of damages after chemical causes have been reported in various conditions. Chemically damaged hairs tend to be rough and dry, and usually lose their color and strength. In our previous studies, chemical causes induced structural damages to all compartments of hair cuticles, especially endocuticle and cell membrane complex (CMC). Permanent waving induced more damages to endocuticle and CMC than hair dyeing process. In this study, we investigated repairing efficacy of 3 general ingredients in hair care products using scanning and transmission electron microscopy and rheometer. Vitamin B5 derivatives (Panthenol) and hydrolyzed protein is the major repairing ingredients in hair shampoo, conditioner and essence. In case of hydrolyzed protein, we used the 2 molecular weights (Mw) of hydrolyzed protein – 350D, 3000D. There are remarkable differences of repairing efficacy between 3 ingredients, especially the changes of cuticle layers and the changes of strength. Repairing efficacy of low Mw hydrolyzed protein is lower than high Mw hydrolyzed protein. We think low Mw protein is able to easily penetration to the hair shaft and easily draw away from hair shaft. In the molecular structure point of view, molecular weight is the most important factor in hair repairing system.

#### **P6: Hair Shaft Damages Induced by Heat**

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The diverse causes of extrinsic hair shaft damage have been documented, and roughly it can be divided into physical causes and chemical causes. Among them, daily damaging causes, especially heat damaging is a well-known cause which is difficult to avoid in daily life. Although heat damage by hair dryer is a severe and important damaging cause, the damaging patterns are not clarified. In our previous studies, hair dryer damages to all compartments of hair cuticles, especially endocuticle. Grossly, damaged hairs by hair dryer tend to be rough and dry, and usually lose their color and strength. In this study, we investigated the damages by hair-iron using scanning and transmission electron

microscopy, hair combing machine, and rheometer. Hair-iron is the general grooming instrument at home to make hair straight. Hair-iron is used directly onto the hair surface so that make the hair damages. After repeating treatment of hair-iron, hair shaft was severe damaged especially hair surface. Hair surface deformation, increasing of hair roughness and color changes were happened. To avoid these damage pattern, protecting is the only way because hair shaft is the dead cell and deformation can not be restored by any treatment.

### **P7: Dynamic Expression Profiling of the Human Dermal Papilla Signature**

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**Key words:** Dermal papilla, hair follicle, induction

Dermal papillae represent a specialized cell niche within the hair follicle, distinct from both dermal sheath and interfollicular dermal fibroblasts. Upon culturing dermal papillae, the gene expression signature of dermal papilla cells is dramatically altered, as evidenced by the observed loss of inductive potential in rodent dermal papilla cells at higher passages. In this study, our aim was to establish the kinetics of gene expression changes in human dermal papillae during culture. We isolated dermal papillae from the scalp skin of three male humans. RNA was extracted from the freshly isolated dermal papillae, from early explant cultures, and dermal papillae cells in culture from passages 1 through 5, all established from the same three donors. Using microarray hybridization to compare the dermal papillae cells from the three subjects, we identified changes in gene expression between freshly isolated human dermal papillae, early explants in culture, and those cultured from passages 1 and 5. We found a large number of transcripts whose expression was altered significantly during the initial explant stage of dermal papilla cell culture whilst surprisingly few further changes were observed after passaging. Genes whose expression was lost during culture included BMP, Wnt and FGF signaling antagonists. One such examples is the BMP and Wnt signaling inhibitor, *Wise*, which was downregulated 198-fold in explanted dermal papillae. We postulate that the abrupt change in the expression signature between intact dermal papillae and papilla cells in culture may reflect an early loss of inductive potential of human dermal papilla cells *in vitro*.

### **P8: The Changes of Integral Hair Lipids After UV Irradiation on 3 Ethnic Hairs**

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**Key words:** Integral hair lipid

Human hair is composed by keratins, water, lipids, pigments, trace elements. Ethnic hair studies have been focused on keratins because of main constituent of hair. Integral hair lipid is different from sebaceous lipid. It was discovered that the lipid in the hair follicle was mainly distributed in hair cuticle and keratinized inner root sheath. Integral hair lipid plays a key role in the maintenance of hair integrity including hydrophobicity and stiffness. Ultraviolet are known to damage hair. Dryness, rough surface texture, decreased luster of hair are caused by UV irradiation. Photochemical degradation of hair result in attack on hair keratin, melanin, lipids. If hair is damaged by UV irradiation, integral hair lipids altered. We conducted study to examine the integral hair lipid changes in 3 ethnic hairs. The range of irradiation of hair irradiated for expectative 6 hours, 24 hours with stimulated ultraviolet ray. The treated hairs showed characteristic morphological damage pattern and integral lipid changes.

### **P9: Prolactin Regulates Keratin Expression in the Human Hair Follicle**

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**Key words:** Prolactin, keratin, hair follicle

Keratins serve crucial roles in hair follicle (HF) physiology and pathology, by providing mechanical support and regulating HF cycling and cell growth. So far, neuroendocrine controls of keratin expression have been sparsely investigated. Since human HFs express functional receptors for prolactin (PRL), and even PRL itself, we studied whether PRL regulates keratin expression in normal human HFs. We treated human scalp microdissected HFs for 6 or 9 days under serum-free organ culture conditions with PRL (400 ng/ml) and/or a pure competitive PRL receptor (PRLR) antagonist. Microarray analysis revealed a large subset of keratins and keratin associated proteins to be regulated by PRL. Using quantitative immunohistomorphometry, PRL upregulated immunoreactivity (IR) of the epithelial stem cells markers keratins 15 and 19. In addition, the IR of keratins 5 and 14, constitutively expressed in the outer root sheath, was upregulated as well. On the other hand, keratin 6 IR was downregulated. All the effects of PRL were blocked by the PRLR antagonist. Interestingly, the latter by itself changed keratin expression, supporting inhibition of the autocrine/paracrine

actions of PRL secreted by HF. Using human K15-promoter-driven GFP labelling, we could demonstrate that GFP-specific IR was upregulated following PRL treatment. These changes in keratin expression were confirmed by qPCR analyses of ORS keratinocytes in culture. In this study we provide first evidence for the potential regulation of PRL on keratin expression in the HF. These results lend further support to the key role that PRL plays in HF growth and function.

### **P10: Thyroid Stimulating Hormone and Thyrotropin Releasing Hormone Regulate Keratins 5 or 6 Expression in the Human Hair Follicle and Epidermis**

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**Key words:** TSH, TRH, keratin, hair follicle, epidermis

The skin and hair are well recognized as targets for thyroid hormones, and patients with thyroid disease demonstrate a variety of hair abnormalities. These effects are mediated at least in part by regulating keratin expression (e.g. keratin 6). Nevertheless, the role of other members of the hypothalamic-pituitary-thyroid axis: i.e. thyrotropin releasing hormone (TRH) and thyroid stimulating hormone (TSH), in human skin and hair biology, is still basically unknown. Recently, we have demonstrated that human hair follicles (HFs) express functional receptors for both TSH and TRH. Therefore, we have studied whether TRH and TSH exert any effect on keratin expression in normal human HF and epidermis. Human scalp skin or microdissected HFs were treated for 5 or 6 days under serum-free organ culture conditions with TSH (10 or 100 mU/ml) or TRH (1-100 ng/ml). Microarray analysis showed a large subset of keratin genes to be regulated by TSH or TRH. By immunohistochemistry, TRH downregulated keratin 6 immunoreactivity (IR) in epidermal and HF keratinocytes *in situ*, while TSH up-regulated keratin 5 IR in epidermal and matrix keratinocytes. TSH also upregulated epidermal keratin 5 mRNA transcript level, as shown by real-time PCR analysis. We provide here the first evidence that TSH and TRH regulate keratin expression in human skin (directly or indirectly), on the gene and protein level.

### **P11: A Case Series of Alopecia Areata in China: A Study of 655 Patients**

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**Key words:** Alopecia areata, quality of life

The clinical features of Alopecia areata (AA) are well documented in different countries, but the investigation of AA and the quality of life in Chinese patients have not been studied adequately. 655 AA patients (320 males, 335 females, aged from 2 to 85 years) who visited our department from 2006 to 2008 were interviewed. The psychological impacts of AA were assessed by Dermatology Life Quality Index (DLQI) questionnaires. 13.7% patients had alopecia totalis and/or alopecia universalis (AT/AU). These patients were younger at the age at presentation and of onset than those with patchy AA ( $P = 0.009$ ;  $P = 0.000$ ), were more likely to have atopic diseases ( $P = 0.022$ ). Duration of patients with AT/AU or in early onset (<16 years) is significantly longer than patchy AA (43.1 vs. 10.3 months) or that for late onset (38.9 vs. 11.9 months). DLQI score ranged from 0 to 29, mean score was  $5.4 \pm 5.5$ . It is higher in patients with AT/AU (8.0 vs. 5.1), in long duration ( $\geq 12$  months) (6.6 vs. 5.1) and with recurrency (6.8 vs. 4.9). Patients with symptoms had a higher score than those without it (7.0 vs. 5.2). There are new characteristics of patients with AA in China. The quality of life was profoundly affected by alopecia areata, especially of the patients with extensive alopecia, long duration, recurrences and local sensations.

### **P12: Dermatology Life Quality Index in 1084 Chinese Men with Androgenetic Alopecia**

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**Key words:** Androgenetic alopecia, quality of life, psoriasis vulgaris

Androgenetic alopecia (AGA) is a common disorder in male. In our previous community-based study, the prevalence of Chinese men was less (19.9%, 2006) than Caucasians. As a biologically benign disease, AGA affects the self-esteem variously. To investigate the influence of AGA, this hospital-based study about the Dermatology Life Quality Index (DLQI) in 1,084 Chinese men was carried out. Type III vertex and IV was the major (61.8%) depending on Norwood-Hamilton scale. The range of age was 17-59 years (mean age  $29.08 \pm 6.432$  years), and the mean age of onset was  $25.65 \pm 5.676$  years. A positive family history was found in 726 subjects (65%). The mean DLQI score for all patients was  $7.47 \pm 5.302$  points. We mainly compared age of onset, duration of onset, educational level and family history. Younger and unmarried subjects were more affected than the rest of patients. However, duration of onset ( $39.96 \pm 31.081$  months), educational level and family history make no difference. We also compared the score with contemporaneous 84 male patients with psoriasis vulgaris (DLQI score  $7.40 \pm 5.897$ ), and impact on the quality of life caused by AGA was similar to psoriasis vulgaris ( $P > 0.05$ ).

### **P13: Hair Growth Parameters of Chinese Subjects in Spring and Autumn**

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**Key words:** China, hair, growth, seasons

Hair growth parameters have been studied most often in Caucasian and African hair. Although several studies have been reported in Korean and Japanese subjects, few data is available on Chinese hair in the literature. To evaluate and compare the hair growth parameters of Chinese subjects in spring and autumn, sixty Chinese volunteers (30 women, 30 men, mean  $\pm$  SD age  $40 \pm 7$  years) living in Shanghai participated in the study. Phototrichograms were performed in order to evaluate the hair growth parameters: hair density, telogen percentage and rate of growth. For each volunteer, three regions of the scalp (vertex, temporal and occipital areas) were assessed. Hair density varied from 57 to 217 hairs  $\text{cm}^{-2}$ , with higher counts on the vertex. Significant differences between two gender groups and season groups were recorded. Telogen percentage of hair greatly varied from 1.1% to 48.1%, with higher levels in autumn, and no difference related either to scalp region or to gender. The rate of growth was variable from 409 to 884  $\mu\text{m day}^{-1}$ , with lower counts on the vertex. Gender difference was shown in spring but not in autumn. No significant difference was found between two season groups. The present study showed low hair density and high growth rate in Chinese subjects as described previously in other Asian populations and demonstrated seasonal changes of hair growth parameters between spring and autumn.

### **P14: Coenzyme Q10 Discloses Positive Effects on Keratin Expression in Human Hair**

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**Key words:** Coenzyme Q10, keratin synthesis, ageing

Ubiquinones are widespread in nature and Coenzyme Q10 (CoQ10) is one of best investigated representative of this group of molecules. In the inner mitochondrial membrane it is involved in a series of enzymatically catalyzed reactions necessary for oxidative phosphorylation via the electron transport chain. Recent studies demonstrated the efficacy of CoQ10 regarding therapy and prevention of heart diseases or neurological disorders. However, the biological effects on skin appendages such as the human hair follicle remain to be elucidated. This lack of knowledge directed us to the here described studies where

we show that CoQ10 also delivers positive effects to the hair follicle. We previously demonstrated that aging processes in the human hair follicle are associated amongst others with a decline in certain hair keratins. Using a reconstructed hair follicle model we identified CoQ10 as a potent bioactive stimulating the gene expression of different hair keratins, especially those which are reduced during aging processes in hair follicles. These results directed us to investigate a shampoo formulation enriched with CoQ10 in a placebo controlled panel study. In a left/right comparison a group of healthy volunteers about 40 years and older daily applied both formulations for four days. Throughout the test period the gene expression of different hair keratins from plucked hair follicles was determined using quantitative PCR techniques. Subsequent statistical evaluation demonstrated an increase of age relevant hair keratins in human hair roots treated with CoQ10, thus pointing out the striking benefits using CoQ10 to fight age related changes in hair.

### **P15: Psoriasis (S100a7) is Expressed in Outer Root Sheath of the Hair Follicles in Human Scalp**

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**Key words:** Psoriasis, hair follicles, outer root sheath

Psoriasis (S100A7) was originally described to be expressed by psoriatic keratinocytes. Its expression has been observed in the upper part of normal epidermis, sebaceous glands and hair follicles. However, the exact localization of psoriasis in hair follicles is not known yet. In this study, we examined the immunohistochemical localization of psoriasis in vertical and transverse sections of paraffin embedded normal scalp skin. In the vertical and horizontal sections of human scalp, psoriasis was expressed in the epidermis, sebaceous glands and outer root sheath (ORS) of the hair follicles. It was expressed in the upper three fourths of ORS. In the upper part of hair follicles, it was expressed in suprabasal layers of ORS. However, in the lower part of hair follicles, it was expressed only in the innermost layer of ORS. In this study, psoriasis was expressed in the differentiated region of the ORS of the hair follicles. This finding suggests that psoriasis may be involved in differentiation process or function as an antimicrobial peptide to protect hair follicles. Further study is necessary to elucidate its function in hair follicles.

### **P16: Capillary Treatments Using siRNA Oligonucleotides: A New Gene Therapy Tool?**

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**Key words:** Hair follicle, melanogenesis, melanocytes, gene therapy, siRNA, cosmetics

Hair loss and premature graying may have dramatic effect on the life of affected individuals. Therefore capillary treatments for changing hair colour and promoting hair growth are continuous challenges in dermo-cosmetic industry. Currently available treatments, apart from having a controversial efficacy, can be very aggressive to hair and underlying skin. Interfering with genes in order to induce changes in colour or growing properties of the hair are hot topics and so far no products are commercially available. In this work, a new, gene therapy-based approach was used for topic capillary treatment using DBA/2J mice as a model. siRNA targeting Keratin 1 (K1), a gene important for hair structural integrity, was delivered to hair follicles to determine if silencing this gene, could be effectively achieved. Hair growth was synchronized through warmed wax depilation of skin patches and different delivery formulations were used for daily applications. *In vivo* phenotypic responses, morphology of hair and post-mortem skin samples were analyzed. No apparent significant effects were observed though additional immunohistochemical, biochemical and expression analyses are being performed to determine the extension of genetic and morphological modifications induced by the treatments.

### **P17: Clinical Experience of Combined Application of L-cystin, Yeast and Pantothenic Acid in Russia**

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**Key words:** Telogen effluvium, pantovigar, treatment, hair loss

Clinical efficacy of the combination of Cystine, Medicinal Yeast, and B Vitamins (Pantovigar®) use in treatment of diffuse telogen effluvium in Russian women population was studied. This open-label randomized study enrolled 40 women of age group from 18 to 45, diagnosed “diffused telogen effluvium”. Women were randomized to two parallel groups – one control and one, who was receiving the study product for 6 months as 1 capsule 3 times per day. Estimation of efficiency is conducted by “blind” study and included dynamic studying before and after treatment overview photos of the scalp, measuring hair density and mean diameter of the hair in standard points of the parietal and occipital areas, determining the number of hairs similar to lanugo, and taking a phototrichogram. To study subjective opinions on the treatment efficacy, 380 Russian women who used the Pantovigar were asked to fill out a questionnaire. After the treatment, Pantovigar group, number of hairs in the telogen phase decreased from 21% to 14% ( $P < 0.01$ ), the mean thickness of hairs in the parietal area increased from

55 to 62  $\mu\text{m}$  ( $P < 0.01$ ), the average number of dysplastic hairs decreased from 24% to 15%. In the control group, the results did not demonstrate statistically significant changes ( $P < 0.05$ ). Respondents indicated hair loss reduction was observed by 88.5%. Of these, 57% of respondents reported that this sign appeared 2 to 3 months after the treatment started. Improvement of hair structure - 76.9%, intensification of hair color –29.3%. The reached clinical effect is declared efficiency.

“Disclosure of Conflict of Interest” The presented study “Clinical experience of combined application of L-cystin, yeast and pantothenic acid in Russia” was performed with financial support of Merz Pharmaceuticals, Russia. The data is not publishes yet.

### **P18: Role of Ornithine Decarboxylase in Human Hair Follicles**

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**Key words:** Ornithine decarboxylase, hair follicle, hair growth

Ornithine decarboxylase(ODC) is a pivotal enzyme in the polyamine biosynthesis, has been shown to be important in cell proliferation and differentiation, including liver, brain etc. Increasing evidence suggests that ODC play an important role in the progression of hair follicle initiation and hair cycling. The purpose of current study is to investigate the role of ODC in human hair follicles. We examined the expression of ODC in the human hair follicles and its expression change in organ cultured human hair follicles. Furthermore, we examined the effect of ODC on human keratinocyte growth using small interfering RNA(SiRNA) technique. Human hair follicles expressed ODC at mRNA and protein level. ODC immunoreactivity can be detected in the epithelium of human anagen hair follicles. During the transformation from anagen to catagen, ODC expression appeared down-regulated. Gene transfection of ODC SiRNA was markedly down-regulated expression of ODC and resulting in suppression of keratinocyte proliferation. Based on our results, we suggest that ODC may play a role in the hair growth and hair cycle change.

### **P19: Clinical Efficacies of Topical Agents for the Treatment of Seborrheic Dermatitis on the Scalp: A Comparative Study**

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**Key words:** Betamethasone, seborrheic dermatitis, tacrolimus, treatment, zinc pyrithione

Previous studies have shown that topical steroid and shampoo containing zinc pyrithione provide clinical benefits for treatment of scalp seborrheic dermatitis. But the clinical efficacy of topical tacrolimus, newly developed calcineurin inhibitor on seborrheic dermatitis is not well investigated yet. We wanted to compare the clinical efficacy of topical tacrolimus with that of conventional treatment (zinc pyrithione shampoo and topical betamethasone) for treatment of seborrheic dermatitis on the scalp. Patients with seborrheic dermatitis on the scalp were randomly allocated to receive topical betamethasone, topical tacrolimus, or zinc pyrithione shampoo. Some patients were instructed to continue the treatments for 8 weeks and the others to discontinue the treatments at week 4. We have evaluated the efficacy using clinical severity score, dandruff score and sebum secretion at baseline, week 4 and week 8. All treatment groups showed significant improvements respectively in clinical assessment after 4 weeks. While the patients treated by zinc pyrithione improved continuously even after cessation of the treatment, the patients treated by betamethasone lotion or tacrolimus ointment were aggravated clinically. Topical tacrolimus was as effective as topical betamethasone, and showed more prolonged remission than topical betamethasone. To treat seborrheic dermatitis on the scalp, we think that the combination therapy of topical steroid or topical tacrolimus, and zinc pyrithione is recommended.

## **P20: The Effects of Dihydrotestosterone on the Dermal Papilla Cells of Balding, Non-balding and Beard**

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**Key words:** Alopecia, DHT, dermal papilla, beard

Dihydrotestosterone (DHT) is famous as the key regulator of expression in human hair growth. They also plays an important role in male-pattern baldness. In human body, circulating DHT enters the hair follicle dermal papilla cells (DPCs) via the capillaries located at the bottom of the follicles. After puberty, frontal (balding) area is miniaturizing, in the other hands, occipital area is not affected at all. Interestingly beard hair and pubic hair are growing much more after adolescence. Each DPCs may have their distinct properties. To investigate this paradoxical character of hair, We cultured each areas in same individual (Frontal, Occipital,

Beard). After that, We treated DHT on each DPCs. Then we isolated RNA of DPCs. We used Human Genome U133A 2.0 gene chips (Affymetrix) and Hugen ST 1.0 chips(Affymetrix) to check gene expression levels. For systems biological approach to analyze them, we used "R" program and R-packages as analysis tool as well as matlab programs. We found some signaling pathway molecules are differentially involved. Some genes are paradoxically affected by DHT. High-throughput data approaches to androgenic alopecia and hair research would widen our knowledge of disease as well as pathophysiologic pathway of male-pattern baldness.

## **P21: Pressure Alopecia**

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**Key words:** Operation time, permanent alopecia, pressure alopecia, temporary alopecia

Pressure alopecia is a non-scarring alopecia that follows prolonged pressure on the scalp. It is a rare but preventable complication that is most commonly observed after surgical procedures under general anesthesia. To describe the clinical manifestations, course and histopathological features, 18 Korean patients, diagnosed as pressure alopecia at our department from 2004 through 2008, were evaluated. The ratio of male to female was 1:1, and the mean age of patients was 45.1 years. Eleven patients (61.1%) had a history of surgical procedures and seven patients (38.9%) had a history of comatose state or immobilizing illness. The mean duration from the time of pressure on scalp to onset of alopecia was 3.7 weeks. This duration was 3.1 weeks in patients with surgery and 4.5 weeks in patients without surgical procedure. The occipital area was the most commonly affected site. Histopathologic examination showed the increased hair follicles of catagen or telogen, dermal fibrosis and vascular changes. Fourteen patients (77.7%) experienced a complete recovery with hair regrowth, whereas four patients (22.3%) developed a permanent scarring alopecia. All patients with permanent alopecia had a pressure on scalp from operative treatment. They showed massive inflammation around hair follicles and upper dermis in biopsy specimen. The mean operation time was 4.7 hours in patients with temporary alopecia and 8.0 hours in those with permanent alopecia ( $P < 0.05$ ). The operation time was the only difference between two groups. These results showed pressure alopecia could progress a permanent cicatricial alopecia and suggested the most significant factor in prognosis might be an operation time.

## **P22: Clobetasol Propionate May Contribute to Improvement of Parameters of Hair Loss in Seborrheic Dermatitis: Preliminary Study**

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**Key words:** Seborrheic dermatitis, clobetasol propionate

Seborrheic dermatitis (SD) is a common chronic relapsing scaly dermatosis. Although it has been known that hair loss is not directly related with SD, many Korean patients believe that SD is one of the causes of hair loss. We investigated the relationship between SD and hair loss. Furthermore, we want to elucidate the subjective and objective improvement of hair loss condition after using clobetasol propionate (CP) 0.05% shampoo. Six moderate to severe SD patients complaint of hair loss were enrolled and they were medicated with CP shampoo. Using with office based hand held phototrichogram program (Folliscope™), hair density and thickness were measured at 0, 4, 8, 12, 16, 20 weeks. Subjective symptoms of itching, burning and tingling were markedly improved according to increment of CP using duration. Objective signs of erythema and greasy scales also similar results. Interestingly, hair thickness, not hair density, was dramatically increased after using CP shampoo at the 16 weeks. Anti-inflammatory action of CP shampoo is powerfully helpful to calm down of scalp SD. This healthy scalp skin condition may contribute to externally looking thick hair growing indirectly.

### **P23: A Case of Successful Treatment of Multifocal Alopecia Areata with Dexamethazone Oral Puls – Therapy Followed by Polyosis**

**Tsikhankouskaya I, Vladimirovna**

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**Key words:** Alopecia areata, dexamethazone, pulse therapy

A 21 years old male patient was diagnosed alopecia areata and treated for several months with topical glucocorticoids. The treatment was ineffective during several months and the hair loss continued. Total percentage of hair loss was estimated as 70 and alopecia areata considered to be subtotalis. The patient presented signs of emotional stress caused by rapid hair loss. Microscopy of the epilated hair showed dystrophic anagen hair. Histology founded degenerative changes of the hair sheaths. A lot of apoptotic keratinocytes where founded in the epidermis between hair follicles. Typical “swarm of bees” lymphocytic inflammatory infiltrate was found around hair follicles. Vessels of hair follicle where practically absent. Taking in account chronic course of alopecia, resistance to local therapy, psychological state of the patient it was decided to administer dexamethazone pulse therapy. The patient took 5 mg of dexamethazone per os during two successive days of every week. The dosage was taken in the morning after food intake. Eight pulses during two months where taken totally. The re-growth of hair began in the 3d week of the treatment. After the course of pulse therapy in two weeks a hair loss was registered

but the intensity was much less than before the therapy. Full re-growth of the white hair was observed in 8 months after the therapy. As the polyosis resisted the patient colored his hair for the better cosmetic outcome

### **P24: Regulation of Melanogenesis by GDNF and Neurturin as a New Candidates in Human Follicular DPC**

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**Key words:** Hair, DPC, GDNF, melanogenesis

Hair and skin colour mostly depend on the proper activity of melanogenic melanocytes. New candidates were reported from both black and white haired dermal papilla cells (DPCs) of the human scalp by cytokine array. These are glial cell line-derived neurotrophic factor (GDNF), and its family member, neurturin (NTN). These are detected more in DPCs from black haired follicles versus white haired follicles. We discovered that the comparative expressions of GDNF and NTN and their cognate receptors were decreased in white haired follicles *in vitro*. We examined a pivotal role of melanogenesis by GDNF and their family members, neurturin (NTN) in murine melanoma cell line, B16F10. Our study confirmed that GDNF and NTN have a great melanogenic potential. Cells were serum-starved for 24 hr, and replenished with GDNF or NTN for indicated times. The samples were harvested and analyzed by zymography for tyrosinase activity and by spectrophotometry for melanin content measurement. The expressions of melanocyte-specific proteins including MITF (microphthalmia transcription factor), TYR (tyrosinase), and TRP1 (tyrosinase-related protein 1) increased by GDNF and NTN treatment. By contrast, there was no change in the expression of DCT (tyrosinase-related protein 2) by treating both molecules. The proliferation of melanocyte was also augmented by GDNF and NTN. Our preliminary findings suggest that hair follicle melanogenesis may be regulated by GDNF and NTN acting via MITF and TYR promoter.

### **P25: Blocking Potassium Channels, a New Route for the Inhibition of Human Hair Growth**

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University of Bradford Centre for Skin Science

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**Key words:** Organ culture, tolbutamide, hirsutism, excessive hair growth, balding

Minoxidil, which opens ATP-sensitive potassium ( $K_{ATP}$ ) channels in cell membranes, is used to treat balding, although its mechanism of action was unclear.  $K_{ATP}$  channels are composed of pore-forming subunits, Kir6.x, and regulatory sulfonylurea receptor, SUR, subunits which are the site of drug interaction. Recently two forms of  $K_{ATP}$  channel were found in human hair follicles: SUR1 and SUR2B. Minoxidil only acts via SUR2B. Tolbutamide, a  $K_{ATP}$  channel blocker, counteracted minoxidil-stimulated hair growth in culture at high concentration. At this concentration tolbutamide can inhibit both channels, but at lower concentrations it is selective for SUR1. Therefore, we aimed to determine whether inhibiting only the SUR1 channel would alter human hair growth *in vitro*. Human scalp follicles were cultured in the presence of tolbutamide (10nM-1mM) in dimethylsulphoxide (DMSO) (0.001%) with insulin and hydrocortisone. Eight follicles were observed, measured and photographed daily for 10 days from each of 5 individuals in each concentration (7). Tolbutamide at 10 nM had no significant effect on the total increase in hair follicle length, growth rate or percentage of follicles in anagen. However, 100 nM, 1  $\mu$ M, 10  $\mu$ M, 100  $\mu$ M and 1mM all significantly inhibited all these parameters. As the SUR1  $K_{ATP}$  channel is inhibited at 2  $\mu$ M, while the SUR2B requires 0.9 mM, this indicates that tolbutamide can inhibit human hair growth in organ culture by acting on two different  $K_{ATP}$  channels. Further investigations of the roles of these two  $K_{ATP}$  channel types in human hair follicles could enable the development of better treatments for both hair loss and excessive hair conditions.

### **P26: Rat Vibrissa Dermal Papilla Cells Cultured in Follicular Keratinocyte-conditioned Media Maintain Hair Inducing Capacity**

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**Key words:** Dermal papilla, follicular keratinocyte-conditioned media, hair inducing capacity, hair follicle regeneration

Dermal papilla (DP) is located at the bottom of hair follicle and plays an important role in hair growth and maintenance of hair cycle. DP cells in early passage are known to maintain proliferation and hair inducing capacity, but these characters are lost in long-term culture. Here we investigated whether DP cells cultured in follicular outer root sheath keratinocyte-conditioned media (ORSCM) maintain hair inducing capacity and, if so, tried to identify genes involved in hair inducing capacity. DP cells grown from explants of isolated rat vibrissa dermal papillae, were maintained in either DMEM or DMEM supplemented with 50% ORSCM. We found that rat vibrissa DP cells cultured in ORSCM-supplemented media maintain proliferation in long-term culture. To examine that DP cells cultured in ORSCM maintain hair inducing capacity, we operated a graft chamber assay. Hair

follicle regeneration was observed only in ORSCM-maintained DP cells. We next screened differentially expressed genes in DP cells cultured in ORSCM. We found that a number of genes are differentially expressed. Differentially expressed genes identified in this study may be useful markers for preservation of hair inducing capacity.

### **P27: Minoxidil Activates $\beta$ -catenin Pathway by Phosphorylation of GSK3 $\beta$ via PKA and PKB in Human Dermal Papilla Cells**

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**Key words:** Minoxidil,  $\beta$ -catenin, GSK3 $\beta$ , PKA, PKB

Minoxidil is a potent trichogenic agent and widely used for the treatment of hair loss. In this study, we examined whether minoxidil activates  $\beta$ -catenin pathway which is required for the maintenance of hair growth phase (anagen) characteristics of dermal papilla cells. First, we examined the activation of  $\beta$ -catenin reactive promoter pTOPFLASH by luciferase reporter assay. Enhancement of reporter activity was observed after treatment with 100  $\mu$ M minoxidil. Next, we observed nuclear  $\beta$ -catenin accumulation by minoxidil treatment as examined by Western blot analysis and immunocytochemical staining. Also, we observed that minoxidil treatment increases phosphorylation of GSK3 $\beta$  which is phosphorylated by several kinases including PKA and PKB. Therefore, we examined whether minoxidil activates PKA and PKB. Indeed, minoxidil increased cAMP concentration and the phosphorylation of PKA. In addition, the level of phosphorylated PKB (Akt) increased significantly by minoxidil treatment. Our data demonstrate that minoxidil inactivates GSK3 $\beta$  by phosphorylation of PKA and PKB, resulting in activation of  $\beta$ -catenin pathway in human dermal papilla cells.

### **P28: A Transcriptional Response to Wnt3a in Human Dermal Papilla Cells**

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**Key words:** Wnt3a, human hair, dermal papilla cells

It is known that dermal papilla *in vivo* is one of the sites of Wnt/b-catenin signaling. It is also known that maintenance of Wnt/b-catenin pathway is required for hair-induction, keeping dermal papilla cells in anagen-phase characteristics. However, little is known about the genes regulated by Wnt/b-catenin pathway in dermal papilla cells. Therefore, we first examined whether DP cells *in vitro* respond to canonical Wnt. Cells

were transiently transfected with the b-catenin responsive TCF reporter plasmid (pTOPFLASH) and corresponding control promoter (pFOPFLASH) to assess the activity of b-catenin signaling by Wnt3a (one of the canonical Wnts). Results showed that Wnt3a significantly stimulates the transcriptional activity of pTOPFLASH but not pFOPFLASH. As we observed DP cells respond to Wnt3a, microarray analysis was carried out using Affymetrix gene chips. We have identified a number of genes that are regulated by Wnt3a. Differential expression of some of the genes was confirmed by RT-PCR analysis. Elucidating the role of Wnt3a-regulated genes would help our understanding of hair-induction and maintenance of anagen phase.

### **P29: Hair Keratins Evolved from Claw Keratins: A Comprehensive Analysis of Cysteine-rich Keratins in Non-mammalian Model Species**

**Leopold Eckhart, Karin Jaeger, Luisa Dalla Valle, Sandra Szabo, Maria Buchberger, Claudia Ballaun, Alessia Nardi, Caroline Stremnitzer, Mino Ghannadan, Lorenzo Alibardi, Marcela Hermann, Erwin Tschachler**

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**Key words:** Keratins, nail, claws, evolution, genetics

The evolutionary origin of hair is unclear because of an incomplete fossil record. However, the evolutionary history of the genes, that determine the structure of hair, can be inferred from the distribution of these genes among modern vertebrates. The main components of hair are the hair keratins which establish the hard properties of hair by inter-molecular crosslinking at multiple cysteine residues. Here we analyzed by comparative genomics the keratin genes of amphibians, reptiles, birds and mammals. Four groups of cysteine-rich keratins were identified: high cysteine type I keratins specific for sauropsids (reptiles and birds), high cysteine type II keratins specific for birds, type I hair keratins and type II hair keratins. Strikingly, the hair keratins were not found in mammals only but also in sauropsids. The lizard, *Anolis carolinensis*, contained two type I and four type II hair keratins, and the chicken contained one type II hair keratin. For studies of protein expression, antibodies were raised against two hair keratin homologs of the lizard. Immunohistochemistry demonstrated that both proteins were specifically expressed in the claws of the lizard. In summary, this study shows that hair keratins are not restricted to mammals and, therefore, must have evolved with an original function that was not associated with hair. We propose that the claws of the last common ancestor of mammals and reptiles were the main sites of hair keratin expression before a change in the expression pattern of these proteins facilitated the evolution of hair.

### **P30: DNase1L2 Has an Essential Role in the Degradation of Nuclear DNA in Hair and Nails**

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**Key words:** DNA degradation, nucleus, nail, endonuclease, genetics

The hair is formed by terminal differentiation of keratinocytes of the hair matrix. In the course of this process nuclear DNA is degraded. We have reported recently that differentiated keratinocytes of the hair, the nail and the epidermis express high levels of deoxyribonuclease 1-like 2 (DNase1L2), an endonuclease specific to the skin. To assess the physiological role of DNase1L2, we generated DNase1L2 knockout mice and characterized their phenotype. DNase1L2<sup>-/-</sup> mice were healthy, fertile and macroscopically normal. Histological investigation revealed that the interfollicular epidermis of DNase1L2<sup>-/-</sup> mice was orthokeratotic and morphologically not different from that of wildtype mice. By contrast, all hair types investigated as well as the nails of the knockout mice differed strongly from those of wild-type mice. Virtually all corneocytes of the hairs and nails of DNase1L2<sup>-/-</sup> mice contained nuclear remnants that were strongly labeled with the DNA dye Hoechst 33342. DNA extraction and quantification by real time PCR showed that hair of knockout mice contained several hundred-fold more genomic DNA than hair of wild-type mice. Since the content of mitochondrial DNA was the same in hair of knockout and wild-type mice, DNase1L2 activity appeared to be restricted to the nuclear compartment. Taken together, these data show that DNase1L2 is essential for the breakdown of genomic DNA during hair formation and identify DNase1L2 as the first specific mediator of differentiation-associated keratinocyte cell death in vivo. The DNase1L2 knockout mouse will be used to uncover the physiological role of nuclear DNA removal in the hair.

### **P31: Prostaglandin F<sub>2α</sub> Can Act Directly on Human Hair Follicles: Scalp Follicles Increase Growth in Organ Culture and Express Receptor Genes**

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**Key words:** Prostaglandin F<sub>2α</sub>, latanoprost, organ culture, FP receptor, new treatments

A prostaglandin F<sub>2α</sub> analogue, latanoprost, stimulates eyelash growth when used to treat glaucoma (ocular hypertension).

How prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) increases eyelash length and pigmentation is unknown; possibilities include direct action on follicles or increasing follicular blood flow. To determine whether human hair follicles can respond directly, we investigated the effect of  $PGF_{2\alpha}$  on scalp follicles in organ culture and the gene expression of the  $PGF_{2\alpha}$  receptor, FP. Scalp skin from non-balding areas was obtained from healthy individuals undergoing cosmetic surgery. For organ culture, skin was collected into sterile tubes in medium containing antibiotics and for reverse transcriptase-polymerase chain reaction (RT-PCR) into RNA stabilization solution,  $RNA_{later}$ . Hair follicles were individually microdissected and cultured for nine days with daily examination, photography and measurement or pooled for each person for RT-PCR using primers newly designed from the human FP gene and control  $\beta$ -actin primers to ensure cDNA quality.  $PGF_{2\alpha}$  (10nm, 100nm, 1 $\mu$ M) significantly stimulated the percentage of growing follicles, hair follicle growth rate and the total length of hair follicle produced ( $n=5$ ;  $P < 0.01$ ). RT-PCR identified FP gene expression in 5 different follicle samples; sequencing confirmed gene identity. Thus, human follicles respond biologically to  $PGF_{2\alpha}$  in organ culture and express genes for its receptor, FP, suggesting that  $PGF_{2\alpha}$  can act on receptors within human hair follicles to stimulate growth. This suggests that  $PGF_{2\alpha}$  may play a role in normal hair growth and that novel drugs related to  $PGF_{2\alpha}$  could be useful in the treatment of hair disorders.

### **P32: Murine Eyelashes As a Study Model for Hypertrichology or Alopecia of Eyelashes**

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**Key words:** Eyelash, mouse model, trichomegaly

We aimed to develop a murine eyelash model for elucidating eyelash-specific mechanisms of hypertrichology or alopecia. First we undertook intensive morphological investigations to establish the model. Upper eyelids from female C57/BL6 mice of various ages (0-6 weeks) were collected. Eyelids fixed with 4% paraformaldehyde were trimmed and observed under a stereomicroscope and a light microscope. Eyelids fixed with Ito's fixative were embedded in epon resin, sectioned with 1 $\mu$ m thickness, stained with Toluidine blue, and observed under a light microscope. After characterizing murine eyelashes, we applied a trichomegaly-inducing agent, bimatoprost (3 $\mu$ l; Lumigan<sup>TM</sup>, Allergan), and its vehicle for 2 weeks. Murine eyelashes varied in length, thickness, and pigmentation, such as small non-pigmented vellus eyelashes and long well-pigmented eyelashes

with medulla structures. The number of eyelashes was increased by 3 week after birth and stayed the same for at least 6 weeks. The morphogenesis took a similar feature and time course to that of dorsal skin hairs, followed by a synchronized growth cycle progressing faster than dorsal skin hairs. Each follicle had only one completed eyelash, suggesting that exogen occurs in every cycle. Bimatoprost application induced trichomegaly, which was not associated with a new follicle formation. More follicles presented anagen phase and the bulbs of early anagen were thicker, but morphological abnormalities were not observed. In this study, we characterized murine eyelashes, which respond to a trichomegaly-inducing agent in a similar manner to human eyelashes. Our data provide useful information on murine eyelashes as a study model for hypertrichology or alopecia of eyelashes.

### **P33: A Comparison of Caucasian and Korean Hair Density in Occiput**

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**Key words:** Hair density, ethnic profile, hair restoration surgery, androgenetic alopecia

Numerous investigators have estimated hair density over the years. It is generally believed that Caucasian have more hair follicle than Asian. But, no one has compared Asian hair densities with Caucasian using similar objective methods. The purpose of this study is to compare hair and follicular density in a finite surface area in both Korean and Caucasian populations using the same method of measurement. 64 Caucasian and 30 Korean androgenetic alopecia patients who were plan to receive hair restoration surgery with follicular unit extractor (FUE) were enrolled. The occiput (Donor area) of patient was divided into 14 regions of a specified size using a donor area template and the density of hairs and follicular groups in each region was recorded and compared. Caucasian hair density and follicular density are higher than in Korean people (193.07 vs. 165.29/cm<sup>2</sup>, 81.37 vs. 74.81/cm<sup>2</sup>), but the difference is not as great as previously reported (83~122/cm<sup>2</sup>, 55~64/cm<sup>2</sup>). Some doctor said that FUE is not suitable for Asian because of far less density than Caucasian, but this study re-confirms that FUE are not inferior to strip methods in Asian patients.

### **P34: Red Deer (Cervus Elaphus) Hair Follicles Express the Genes for at Least Two Forms of Potassium Channels Like Human Hair Follicles in Anagen, Not in Telogen**

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**Key words:** Hair, gene expression, red deer (*Cervus elaphus*), potassium channels, animal models

Hair loss is psychologically distressing, but not well controlled. Minoxidil, currently the main, but limited, topical treatment, opens ATP-sensitive potassium ( $K_{ATP}$ ) channels in cell membranes. Red deer hair follicles offer a model system for studying hair growth *in vitro* as they are large follicles, readily available without ethical constraints. Various  $K_{ATP}$  channel openers and blockers alter deer follicle growth in organ culture, suggesting the presence of  $K_{ATP}$  channels. This study aimed to validate the deer follicle model further by investigating the expression of  $K_{ATP}$  channel subunit genes in deer hair follicles and to determine whether this varied during the hair cycle. Individual hair follicles were microdissected and pooled from each individual for identification of 5  $K_{ATP}$  channel subunits by RT-PCR. Gene identity was confirmed by sequence analysis. Four  $K_{ATP}$  channel subunits were detected in deer anagen follicles, SUR1, SUR2B, Kir6.1 and Kir6.2; SUR2A was not expressed, although detected in the deer skeletal muscle. No  $K_{ATP}$  channels were detected in telogen follicles. These results indicate that deer hair follicles express the genes for at least two  $K_{ATP}$  channels, only one of which would respond to minoxidil (SUR 2B). This mirrors the situation in human anagen hair follicles, strengthening the use of deer hair follicles as a model system. The absence of gene expression in telogen follicles supports the importance of  $K_{ATP}$  channels in normal anagen processes. Further investigations using deer hair follicles should aid our understanding of hair follicle biology and enable the development of better treatments for hair disorders.

### **P35: Hepatocyte Growth Factor Family Genes Are Expressed in Normal Human Follicles but Reduced in Androgenetic Alopecia**

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**Key words:** Human hair follicles, androgens, androgenetic alopecia, HGF, MSP, c-Met, RON, RT-PCR, dermal papilla, dermal sheath, hair matrix

Androgens are the main modulators of human hair growth, stimulating much hair growth e.g. beard, while inhibiting scalp follicles causing balding. Androgens probably act by altering paracrine regulatory factors produced by the dermal papilla. Since hepatocyte growth factor (HGF) family members, HGF and macrophage stimulating protein (MSP) are implicated in

hair growth, they seem candidates for androgen modulation. We investigated whether and where these genes were expressed in human hair follicles and whether their expression was altered in balding. Scalp skin from healthy individuals was transported in RNA stabilization reagent, RNA<sub>later</sub> for RT-PCR and media for cell culture. Individually microdissected hair follicles (n=5) expressed genes for MSP and its receptor, RON, and HGF and its receptor, c-Met. Sequencing confirmed gene identity. When bulb components were isolated separately (n=3), the dermal papilla and dermal sheath expressed MSP, RON, HGF, and c-Met, while the bulb matrix expressed MSP and RON. In balding follicles (n=3) no MSP was detected. Normal cultured dermal papilla cells expressed HGF, but balding cells only slightly (n=5). Thus, human hair follicles express the genes and receptors for 2 HGF family members. The expression of both factors and their receptors by normal dermal papilla and sheath, while the epithelial bulb matrix cells only produce MSP and RON indicates an autocrine dermal role for HGF. Balding follicles and dermal papilla cells express less MSP and HGF suggesting these paracrine factors are involved in producing large hair follicles. Increasing the levels of these factors could stimulate hair growth in balding.

### **P36: The Involvement of mTOR Signalling Activation in Hair Cycle and Bimatoprost-induced Trichomegaly**

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**Key words:** mTOR, bimatoprost, trichomegaly, hair cycle

Mammalian target of Rapamycin (mTOR) is a central regulator of cell growth by acting as a sensor for ATP and amino acids. Bimatoprost is a prostaglandin F<sub>2</sub>  $\alpha$  analogue, which triggers trichomegaly (elongation, thickening, darkening of eyelashes) in patients under glaucoma therapy. The precise mechanism of bimatoprost action remains to be elucidated. We hypothesized that bimatoprost induces trichomegaly via activation of the mTOR signaling pathway. Dorsal skin of C57/BL6 mice was used to evaluate mTOR activation by immunostaining using an antibody against an activated form of mTOR ( $\alpha$ -pmTOR) in hair follicles at different phases. Then the effect of bimatoprost treatment on mTOR activation was evaluated. In all phases of the hair cycle, pmTOR was expressed at the bulge region of hair follicles and Stratum germinativum of epidermis. In telogen hairs, both the number of pmTOR positive cells and intensity of the staining were low. In anagen hairs, pmTOR positive cells were increased and expression was extended to the entire outer root sheath (ORS) in addition to the bulge region. In catagen hairs, nearly all cells in ORS were pmTOR positive.

Bimatoprost treatment induced elevated number and intensity of pmTOR immunostaining. Our results indicate that: 1) hair cycle progression and activation of mTOR have a correlation, and 2) Bimatoprost may influence hair cycle via mTOR activation.

### **P37: Thyroid Stimulating Hormone and Thyrotropin Releasing Hormone Regulate Keratins 5 or 6 Expression in the Human Hair Follicle and Epidermis**

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**Key words:** TSH, TRH, keratin, hair follicle, epidermis

The skin and hair are well recognized as targets for thyroid hormones, and patients with thyroid disease demonstrate a variety of hair abnormalities. These effects are mediated at least in part by regulating keratin expression (e.g. keratin 6). Nevertheless, the role of other members of the hypothalamic-pituitary-thyroid axis: i.e. thyrotropin releasing hormone (TRH) and thyroid stimulating hormone (TSH), in human skin and hair biology, is still basically unknown. Recently, we have demonstrated that human hair follicles (HF) express functional receptors for both TSH and TRH. Therefore, we have studied whether TRH and TSH exert any effect on keratin expression in normal human HF and epidermis. Human scalp skin or microdissected HF were treated for 5 or 6 days under serum-free organ culture conditions with TSH (10 or 100 mU/ml) or TRH (1-100 ng/ml). Microarray analysis showed a large subset of keratin genes to be regulated by TSH or TRH. By immunohistochemistry, TRH downregulated keratin 6 immunoreactivity (IR) in epidermal and HF keratinocytes *in situ*, while TSH up-regulated keratin 5 IR in epidermal and matrix keratinocytes. TSH also upregulated epidermal keratin 5 mRNA transcript level, as shown by real-time PCR analysis. We provide here the first evidence that TSH and TRH regulate keratin expression in human skin (directly or indirectly), on the gene and protein level.

### **P38: Prolactin Regulates Keratin Expression in the Human Hair Follicle**

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**Key words:** Prolactin, keratin, hair follicle

Keratins serve crucial roles in hair follicle (HF) physiology and pathology, by providing mechanical support and regulating HF cycling and cell growth. So far, neuroendocrine controls of keratin expression have been sparsely investigated. Since human HF express functional receptors for prolactin (PRL), and even PRL itself, we studied whether PRL regulates keratin expression in normal human HF. We treated human scalp microdissected HF for 6 or 9 days under serum-free organ culture conditions with PRL (400 ng/ml) and/or a pure competitive PRL receptor (PRLR) antagonist. Microarray analysis revealed a large subset of keratins and keratin associated proteins to be regulated by PRL. Using quantitative immunohistomorphometry, PRL upregulated immunoreactivity (IR) of the epithelial stem cells markers keratins 15 and 19. In addition, the IR of keratins 5 and 14, constitutively expressed in the outer root sheath, was upregulated as well. On the other hand, keratin 6 IR was downregulated. All the effects of PRL were blocked by the PRLR antagonist. Interestingly, the latter by itself changed keratin expression, supporting inhibition of the autocrine/paracrine actions of PRL secreted by HF. Using human K15-promoter-driven GFP labelling, we could demonstrate that GFP-specific IR was upregulated following PRL treatment. These changes in keratin expression were confirmed by qPCR analyses of ORS keratinocytes in culture. In this study we provide first evidence for the potential regulation of PRL on keratin expression in the HF. These results lend further support to the key role that PRL plays in HF growth and function.

### **P39: Association Analysis of Alopecia Areata and Female Pattern Hair Loss with the Corneodesmosin Gene**

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**Key words:** Alopecia areata, corneodesmosin, female pattern hair loss, genetics

Corneodesmosin (CDSN) is known to be important in the physiology of normal skin and hair and a nonsense mutation in the CDSN gene has previously detected in families with hypotrichosis simplex. In this study, we sought to determine whether variants in the MHC CDSN gene may confer susceptibility to the common hair disorders alopecia areata (AA) and female pattern hair loss (FPHL). 168 patients with alopecia areata and 115 patients with female pattern hair loss were included in the study. Genotyping was also performed in 198 normal control subjects. Allelic discrimination of the CDSN single nucleotide polymorphism (rs2302399) was performed by PCR-RFLP. Homozygotes for

the rare C allele were significantly reduced in frequency in the AA patients compared to both normal controls and the patients with FPHL ( $P < 0.001$ ). In contrast, allelic frequencies in the FPHL were similar to the controls. These results suggest an interesting difference in the pattern of MHC genetic susceptibility between alopecia areata and female pattern hair loss. The pathogenetic significance of this observation for alopecia areata is uncertain but the possibilities include a functional role for corneodesmosin in alopecia areata or alternatively, the association may be due to linkage disequilibrium with other loci in the MHC region such as HLA genes.

#### **P40: Plasticity of Hair Follicle Stem Cells in Wound Repair and Tumorigenesis**

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**Key words:** Lgr5, Hedgehog, BCC, wound, lineage tracing, transgenic mouse models

“Tumors are wounds that do not heal,” states a famous hypothesis formulated by H. Dvorak in 1986. These two processes are prominent in the epidermis, which is exposed to continuous impacts from the surrounding environment resulting in wounds and cellular damage. Hair follicle (HF) stem cells (SCs) contribute to the interfollicular epidermis (IFE) after wounding and are thought to be the cells of origin of the basal cell carcinoma (BCC). Here we show that anagen Lgr5+/CD34- HF SCs and their progeny directly contributed to wound re-epithelialization, demonstrating the importance of anagen HF SC in acute wound repair. Further, lineage tracing of Lgr5+ cells revealed that BCC-like lesions arising in the IFE and the HF have two different cells of origin and that the BCC may represent a polyclonal tumor. All developing basaloid lesions - IFE and HF derived - expressed Lgr5, supporting the idea that IFE cells adopt a HF SC nature during the BCC formation process. Moreover, wounding increased the pathological response of epithelial cells to oncogenic Hedgehog signaling suggesting a convergent nature of these two processes. Both wound healing and BCC formation induced active migration of the Lgr5 progeny out of the HFs. In the latter case the migratory cells do not integrate into forming BCCs but differentiate into keratinized layers. Surprisingly, wounding in GLI1-expressing epidermis induced the formation of Lgr5-traced lesions in the wound area, suggesting that the wound environment can change hair follicle cell fate.

#### **P41: Caffeine – Modulator of a CRH-induced Stress-response in Human Organ-cultured Hair Follicles from Men with Androgenetic Alopecia**

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**Key words:** CRH, stress, caffeine, hair follicle

Human hair follicles (HF) show a local equivalent to the systemic stress-involved hypothalamic-pituitary-adrenal (HPA) axis with corticotropin-releasing hormone (CRH) as its pivotal key stress mediator. CRH inhibits human hair growth *in vitro*. Since stress can be a deterioral factor in androgenetic alopecia (AGA) *in vivo*, it has been hypothesized, that CRH might induce this stress-response in HFs from men with AGA *in vitro*. Moreover, it was tested whether this might be counteracted by caffeine. Extracted HFs from scalp skin biopsies from the balding vertex area of men with AGA were organ-cultured over 120hrs in four different conditions: control (Williams E medium), CRH (10-7 M), CRH (10-7 M) in combination with caffeine (0,001% or 0,005%). CRH suppressed hair shaft elongation, induced catagen, inhibited matrix keratinocyte proliferation and induced apoptosis while caffeine inhibited these effects. CRH and caffeine had contrary effects on the catagen inductor TGF- $\beta$ 2 and the growth factor IGF-1. The investigation of HPA-axis dependent elements showed that CRH significantly up-regulated the three following mediators: its own receptor CRH-R1/R2, the intracellular second messenger IP3-receptor and the ACTH-dependent melanocortin-receptor-2. Caffeine inhibited the immunoreactivity of CRH-R1/R2 as well as IP3-R significantly and MC-R2 moderately. HPA-axis independent stress parameters like substance P and TrkA were also involved in the CRH-induced stress-response. Caffeine inhibited the immunoreactivity of substance P, whereas the anti-apoptotic receptor TrkA was increased. The data provide a caffeine-mediated neutralizing effect on CRH-stressed human HF. This might be an interesting clinical approach to improve stress-associated worsening of androgenetic hair loss by caffeine.

#### **P42: Female Pattern Hair Loss and Severe Hyperandrogenism**

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**Key words:** Female pattern hair loss, hyperandrogenism

A 60 year-old woman presented with a 4 year history of scalp hair loss and mild hirsutism. She was otherwise well. Examination showed a diffuse reduction in hair density, most marked over the mid- and frontal scalp, with preservation of the frontal hair line. The appearance was typical of female pattern hair loss (FPHL) of Ludwig grade II. Investigations revealed a greatly elevated

serum testosterone of 17.8 nmol/L (normal range 0.5-2.6). Laparoscopy showed a 14mm tumour in the right ovary. She underwent bilateral salping-oophorectomy. Histology showed a benign steroid cell tumour (a rare tumour postulated to originate from adrenal cortical rest cells). Following surgery her serum testosterone fell to 0.4 nmol/L. One year post-surgery her hirsutism has improved but there has been no obvious change in scalp hair density. Androgen-secreting tumours are a rare cause

of hyperandrogenism and are conventionally associated with marked virilisation. This case demonstrates that the presentation can be more subtle. It also shows that the pattern of hair loss in women is not a reliable guide to aetiology. Despite a serum testosterone seven-fold about the normal range she showed a typical female pattern of hair loss, rather than a male pattern. This has also been our experience in women with less severe degrees of androgen excess.