POSTER PRESENTATIONS

P-01

Clinical and ultrastructural study of a case of Olmsted Syndrome (OS)

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We studied a 9 month old boy with palmoplantar keratoderma, periorificial keratotic plaques, treated at first as a Zinc deficiency without response. OS was diagnosed. This sporadic entity is caused by a heterozygous mutation in TRPV3 gene, chromosome 17p13.2. Genetic study is in course. Histopathology: We observed psoriasiform hyperplasia, granular layer (GL) with few granules, parakeratotic hyperkeratosis, high nucleus/cytoplasmic ratio, even in suprabasal layers (SBL). Ultrastructure: Basal zone discloses: a normal basal membrane, hemidesmosomes with thick clumps of keratin filaments (KFs), anchoring in inner and external large hemidesmosomal plates. Degenerative keratinocytes, some basal undifferentiated cells were found. Multiple nucleoli and mitosis were seen even in SBL. Glycogen and other organelles were scarce. Extracellular space was augmented, with clusters of isolated desmosomes. A dense mosaic of KFs is seen in upper layers. Numerous desmosomes surrounded by dense material are seen in plasma membrane contiguous to GL. Ultrastructural features are in agreement with augmented mitotic activity and cytokeratin alterations already published. A strong attachment between layers in mucous stratum, may contribute to hyperkeratosis and could be explained by changes here described in desmosomes and hemidesmosomes. Infections and carcinomatous lesions could complicate hyperkeratotic plates of this rare disease. The study of more cases of OS will lead to improvement of our knowledge for better treatment and prognosis.

P-02

Endemic Kaposi sarcoma. Role of genetic tests. A case report

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Kaposi sarcoma (KS) is a low grade vascular tumor associated with Kaposi sarcoma herpes virus/human herpes virus 8 (KSHV/HHV8) of the gamma virus subfamily notable for causing tumors. Epidemiologic-clinical forms of KS include: classic or sporadic which occurs in elderly person of Jewish or mediterranean backgrounds, the endemic form described in indigenous Africans, the epidemic form associated with HIV infection and forms of acquired immunosuppression (organ transplantation and chemotherapy). Case report: A 25 year old black woman migrant from subsaharian area presented with reddish, purple lesions on the distal leg that had been evident since ten months evolving from early macules into plaques that grow into larger nodules. No systemic symptoms were present. Routine laboratory tests were in the normal range, serologic tests for HIV infection and immunohistochemical marker Latency - Associated Nuclear Antigen HHV8 were negative. The differential diagnosis included many vascular neoplasias, infections, inflammatory processes (vasculitides and granulomatous diseases). Two histological examinations during a four month follow up were borderline between KS and its mimics. Tissue sections were taken and Polymerase Chain Reaction (PCR) targeting HHV-8 DNA sequences confirmed a diagnosis of endemic KS. This clinical form usually has an indolent course and appears less refractory to radio- and chemotherapy, however it can compromise the quality of life for causing pain, disfigurement and disability. Conclusions: Caution should be taken not to diagnose KS solely on the basis of immunohistochemical phenotype, in such case a PCR targeting HHV-8 DNA detection appears to be a better diagnostic tool.

P-03

Human Cutaneous Membrane Basal Zone (HCBMZ) - ultrastructural terminology revisited

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Ultrastructural approach of the HCMBZ could be difficult because terms actually in use are confuse. We review in brief the terminology and suggest some modifications (quotation marks) as follows. The basal keratinocyte unit membrane has a dense cytoplasmic leaflet, an electron-lucent intermediate layer and a thinner dermal electron dense leaflet (M line). The ''outer plasmalemmal hemidesmosomal plate'' (20–40 nm, PHP) was called attachment plate because in tangential sections, tonofilaments resemble to reach it. PHP is separated by a narrow electron-lucent space from a thinner ''inner hemidesmosomal plate''; place were usually tonofilaments are really attached. The basal lamina (BL) or lamina densa is fixed to the plasmalemma by the ''sub-plasmalemmal'' lamina lucida, sometimes both were called together as basal membrane. In the middle aspect of the lamina lucida, facing each hemidesmosome, a small electron dense layer is seen: the H line, ''supra-basal'' dense plate or half plate. Anchoring filaments are thin perpendicular filaments that cross this structure. In order to avoid confusion with the next term described, we propose to call them anchoring ''thin filaments''. In the sub-basal region we recognize ''dermal'' anchoring fibrils (collagen = col VII). They descend from BL into papillary dermis, surround collagen (col I-III) and end in anchoring plates (col IV–VII). We suggest to call them ''dermal anchoring plates'' to differentiate from the cytoplasmic plate mentioned. We expect that these little changes suggested could contribute to facilitate the comprehension of the HCMBZ ultrastructure and encourage beginners to do electron microscopic skin studies.

P-04

Ultrastructure of desmosomes as a diagnostic clue in a case of congenital skin fragility syndrome

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We report the case of a girl presenting with fragile skin syndrome from birth, and later on alopecia and focal hyperkeratosis. At age 2 the skin fragility persisted, the hair was thin and sparse and pachyonichya was evident. The patient showed no signs of cardiomyopathy. The differential diagnosis at that stage included pachyonychia congenita, ectodermal dysplasia syndrome and desmosomal gene defects, all of which are caused by a multitude of different mutations on different genes. A skin punch biopsy was taken for transmission electron microscopy. This showed throughout the epidermis a pathological cytoskeleton, with the keratin intermediate filaments clumped around the nucleus and ending abruptly at some distance from the desmosomes. The desmosomal plaques and the other cell organelles had normal ultrastructure. There were no major defects of keratohyalin. This observation further directed the molecular analyses on the desmosomal genes including desmoplakin. Direct sequencing of DNA extracted from peripheral blood lymphocytes showed compound heterozygous mutations in exon 23 and exon 24 of desmoplakin gene, *DSP*. Both mutations lead to frameshift-induced premature terminations in desmoplakin synthesis. Immunhistochemistry confirmed the lack of desmoplakin staining in the patient's epidermis. This case highlights the use of electron microscopy in directing further molecular analyses in the rare cases presenting with skin fragility syndrome. Given the known risk for development of cardiomyopathy in patients with desmoplakin defects, a close cardiac monitoring is planned for this patient. The knowledge that the parents were heterozygous for one desmoplakin gene mutation each could also add in parental counseling.

P-05

The skin as a ''spectrum'': Raman spectroscopy for *in-vivo* diagnosis

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Over the past two decades the Raman spectroscopy, a vibrational spectroscopic technique which gives information about molecular structure with a high level of specificity, is begging to be recognized as a high potential technique for the non invasive study of biological samples.

In dermatological research it can be used as an efficient tool for examination of skin's biochemical structure and composition, aspects of interest for dermal application of cosmetic and pharmacological agents. Raman spectroscopy has also been demonstrated to potentially provide an accurate diagnosis by distinguishing basal cell carcinoma from surrounding normal tissue.

Our objective is to study spectra of the skin layers from which we can obtain information regarding its composition. Surface point measurements and in-depth profiling were performed on *ex-vivo* human skin, to monitor the relative concentration modifications of the major constituents (keratin, urea, water, …) in the thickness of the epidermis. Analyses have been carried out with a near infrared laser (785 nm) by means of a confocal micro-Raman system.

Some preliminary spectra of the epidermis and dermis were collected with particular attention to the filaggrin spectrum, a key protein of several skin diseases like psoriasis and atopic dermatitis. The typical Raman bands of ceramides were detected at 1086 and 1127 cm^{-1}.

The 1655 cm^{-1} peak is attributed to keratin and Raman features of the collagen are identified in the spectrum at 855 and 936 cm^{-1}. Such information is of major interest for the development of *in vivo* diagnosis of skin diseases and the improvement of transdermal drug administration.

P-06

Keratinocyte restricted deletions of Rho A and Rac1 do not substantially alter the ultrastructure of the interfollicular epidermis in mice

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RhoA and Rac1 are two members of the Rho GTPase family of proteins. Rho GTPase proteins can act as molecular switches by cycling between an inactive and an active state. In their active, GTP-bound, state they can function as signalling molecules by binding and activating specific effector proteins. This can control several biological activities including the reorganisation of the actin cytoskeleton. Many *in vitro* studies have therefore linked these proteins to the formation and stabilization of cell-cell junctional complexes as well as to a stable cellular interaction with the epidermal basement membrane, suggesting them as essential factors to the structural integrity of the skin epithelium. We have carried out an ultrastructural analysis of various aspects of the interfollicular epidermis of mice carrying a deletion of either Rac1 or Rho A which, in skin, was restricted in keratinocytes. Transmission electron microscopic (TEM) analysis was carried out in Galway, whereas the tissue was provided by the Brakebusch Lab (BRIC, Copenhagen, Denmark), where housing, treatments, and sacrifice of the mice, took place according to the local animal ethics guidelines and with corresponding ethical approval. TEM analysis included qualitative study and quantitative analysis of the integrity of the DEJ. The data of our investigation support the notion that *in vivo* these proteins, at least when deleted individually, are dispensable for maintaining the integrity of the ultrastructure of the interfollicular epidermis.

P-07

Pigmentation in lichen ruber planus: dermoscopic and microscopic interplay

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Lichen ruber planus (LRP) is a subacute or chronically progressive papulosquamous disorder which might be accompanied by epidermal and dermal pigment lesions. Dynamics and spectra of pigmented lesions which sometimes tend to change along with regression of inflammatory process are poorly understood. The authors aimed to correlate dermoscopic and microscopic findings in patients with long-term LRP condition and pigmentation. A prospective study was performed at the Clinical Centre for Skin and Sexually Transmitted Diseases, Riga, Latvia between September 2012 and April 2013. Inclusion criteria were determined by a clinical diagnosis of LRP and no therapy used at least for 28 days. The nonpolarized digital dermoscope and light microscope were used for pigmented structure investigation in active lesions. Surface microscopy analysis was repeated after two weeks of therapy. A pepper-like, grey pigmentation was the most significant dermoscopic finding, whereas, a dotted brownish pigmentation was a common finding in a sustained process. Furthermore, we found a significant increase of dotted pigmentation after two weeks of therapy in patients with long-term LRP condition. Clinically, in most of the cases epidermal pigmentation remained or even slightly increased along with decrease of inflammatory process. Microscopically, LRP was characterized by basal layer vacuolization, acanthosis, hypergranulosis and lymphocytic infiltrate localized at the dermal-epidermal interface. In old plaques decrease of infiltrate density was accompanied by increase of melanophages. Dermoscopic evaluation of pepper-like, grey and diffuse vs. spotted darker pigmentation might be useful in assessing prognosis in LRP. Darker pigmentation is frequently observed in LRP patients in Latvia. We can assume that this might be related to the patient's skin type and a long inflammatory affection period prior consultation of a specialist. Jointly implemented dermoscopic and microscopic analysis may be helpful in diagnostic assessment and prediction of the course of LRP.

P-08

Parapoxvirus infection in Austria: diagnosis and zoonotic potential - a survey

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Human parapoxvirus infection, a rare, self-limiting, zoonotic disease, often lacks for a reporting system. The genus Parapoxvirus includes nine species, only three of them are said to infect humans: *Bovine papular stomatitis virus, Orf virus* and *Pseudocowpox virus*. Human infections generally arise from direct contact with wild and domestic ruminants. A rapid differential diagnosis is important because skin lesions can resemble potentially life-threatening zoonotic infections, including tularemia and cutaneous anthrax. Skin lesions, progressing through a typical pattern of erythema, macula, papula, and scab, generally appear 5–6 days after infection. In 2010, 2012 and 2013, a woman from a cattle farm, a farmer from a sheep farm and a hunter were hospitalised because of large local finger lesions. The female patient later came down with a generalized rash of pustules similar to that reported in a human case in 2009. The necrotic biopsy material, the swab from the vesicle fluid and the pustules as well as skin lesions of the infected chamois killed by the hunter were sent for laboratory diagnosis. EM diagnosis yielded parapoxvirus infection in negative staining technique within one hour after sample arrival. The biopsy material of the rash pustules was poxvirus-negative. Molecularbiology, PCR specific for the B2L gene and sequence analysis, confirmed the accurate ultrastructure diagnosis. Sections of epidermal lesions showed swellings of keratinocytes which resulted in ballooning degeneration, central cytoplasmic lysis, vacuolation and nuclear pyknosis. Sequence analysis of the parapoxviruses from infected humans and animals showed the spectrum of zoonotic potential of parapoxvirus strains in Austria.

P-09

Pox virus infections of the skin – electron microscopy as a critical diagnostic tool

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Two cases of skin infections with pox viruses are presented. In both cases, the correct diagnosis was corroborated by electron microscopy of negatively stained specimens from scrapings of skin lesions. (1) A hunter from a nearby Tyrolean valley presented with typical lesions on his finger after disemboweling a chamois and, a week later, a marmot. Negative stain showed unequivocal parapox virus particles (Orf virus; *Ecthyma contagiosum*), thus confirming the clinical diagnosis. Molecular analyses disproved an initial suspicion that the virus strain might have been a novel strain specific to deer and contracted from cattle, recently described in the NEJM (363: 2621, 2010). Rather, genetic similarity was to parapoxvirus from sheep suggesting that chamois have acquired the virus from sheep. Indeed, subsequently, several chamois were found that were infected with the same sheep-derived strain of Orf virus. This highlights the relevance of this way of transmission. (2) A Belgian child (9 yrs) on holiday in the Tyrol presented with lesions on his finger. Confirmation of a parapox virus infection was requested. Instead, negative stain specimens revealed the ultrastructure of orthopox virus particles, rather than parapox. Subsequent serological analyses corroborated the orthopox nature of the infection. An initial suspicion was that the infection might have been contracted from vaccinia baits that had been commonly used in campains in Belgium for preventing the spread of rabies amongst foxes and other animals. However, serological analyses did not reveal reactivity against the recombinant rabies glycoprotein. This rendered the acquisition route via vaccinia baits unlikely. These examples underscore the still indispensable value of ultrastructural diagnosis of unclear viral skin infections. Negative stain specimens are ready for inspection some 20 min after taking the sample from the patient and they allow a quick and unequivocal distinction of important classes of viruses.

P-10

Stratum corneum ultrastructure and function in keratosis pilaris

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Keratosis pilaris (KP) is characterized by keratinous plugging of the follicular orifices on the extensor surfaces of proximal extremities with variable perifollicular erythema. We assessed *Filaggrin* (*FLG*) mutation status, stratum corneum (SC) ultrastructure and function in a cohort of 10 individuals with KP. Although KP is frequently associated with atopic dermatitis, only 3/10 individuals harbored heterozygous *FLG* mutations. Our ultrastructural analysis suggests that KP displays a minor barrier abnormality as demonstrated by lanthanum tracer perfusion into and above the stratum granulosum-SC interface in KP whereas in control epidermis perfusion did not extend beyond the stratum granulosum-SC interface. On the functional level, surface pH, SC hydration and transepidermal water loss (TEWL) did not show differences between KP and controls. However, TEWL was increased over involved body sites if compared to uninvolved skin. These results demonstrate that *FLG* mutations only partially segregate with the KP phenotype and that lesional KP displays a minor permeability barrier abnormality.

P-11

Loss of ELOVL1 enzyme causes lethal skin barrier disruption in mice

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ELOVL1 is a member of the mammalian fatty acid elongases and is involved in the synthesis of very long-chain fatty acids. In this study, we generated *Elovl1* knockout mice and revealed the importance of ELOVL1 in the skin barrier function. The mutant fetuses (*Elovl1*−/−) at embryonic day (E) 18.5 showed lower body weight ($P<0.01$) than wild-type fetuses and died within 12 hours when ejected from the uterus, whereas the wild type survived for more than 24 h. The mutants had significantly higher ($P<0.01$) transepidermal water loss, greater susceptibility to water permeability and faster weight loss than the wild type. Skin histology by light and transmission electron microscopy revealed compact stratum corneum (SC), reduced SC intercellular lipids and deficient epidermal lamellar granule contents. Moreover, scanning electron microscopy demonstrated that the skin surface of *Elovl1*−/− mice at E18.5 was much smoother than that of the wild-type mice. These results highlight the importance of ELOVL1 in epidermal barrier function and its potential as a therapeutic target for skin disorders such as atopic dermatitis and ichthyoses.

P-12

Dermoscopic findings of vascular patterns in amelanotic malignant melanoma on the chin

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A 53-year-old man presented with a pinkish nodule, 8 mm in diameter, on the chin. It appeared as a papule 2 months prior to his initial visit and had grown rapidly. Its surface was smooth without any erosion or ulcer. The dermoscopic observation revealed polymorphous vascular patterns characterized by linear-irregular vessels, dotted vessels and arborizing vessels. Histopathological examination showed nests of atypical cells infiltrating into the dermis, partially connecting to the nests in the epidermis. Immunohistochemical stainings revealed that the cells were positive for S-100 protein and HMB45, and negative for AE1/3, CAM5.2 and GCDFP-15. From these findings, we diagnosed the lesion as amelanotic malignant melanoma.

Using such criteria as the two-step method defined by the Consensus Net Meeting on dermoscopy (CNMD2000), ABCD rule (the 'Asymmetry, Border, Colors, and Dermoscopic structures' criteria), Menzies method or 7-point checklist, it might be difficult to diagnose this case as malignant melanoma. However, focusing on irregular vascular patterns including dotted vessels and linear-irregular vessels in the center of the nodule, amelanotic melanoma could be picked up as one of the most probable differential diagnoses.