Annual Meeting of the Society for Cutaneous Ultrastructure Research
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ABSTRACTS

Editors:
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ORAL PRESENTATIONS  O 1–O 15

POSTERS  P 1–P 3

BEST ORAL PRESENTATION AWARD (ex aequo each Euro 250.-):
O-4  Merkel cell carcinoma diagnosed by EM on formalin-fixed tissue
T Funakoshi\(^1\), T Sato\(^1\), R Hosokawa\(^1\), M Saito\(^1\), M Ohyama\(^1\), T Ogata\(^2\), A Ishiko\(^1\)
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O-15  Kallikrein 8 is involved in skin desquamation through cleaving desmoglein 1 and corneodesmosin
M Kishibe\(^1\), H Takahashi\(^1\), A Ishida-Yamamoto\(^1\), S Yoshida\(^1\), H Iizuka\(^1\)
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BEST POSTER PRESENTATION AWARD (ex aequo each Euro 250.-):
P-1  Vogt-Koyanagi-Harada Disease: a novel ultrastructural study
Christine M. Betts\(^1\), Torello Lotti\(^2\), Francesca Prignano\(^2\)
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P-2  Autosomal recessive congenital ichthyosis: Mutations in ichthyin associated with structural abnormalities in the granular layer of epidermis
J Dahlqvist\(^1\), J Klar\(^1\), I Hauser\(^2\), I Anton-Lamprecht\(^2\), M Hellsström Pigg\(^1\), T Gedde-Dahl Jr\(^2\), A Ganemo\(^4\), A Vahlquist\(^4\), N Dahl\(^1\)
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Footnote:
1 The whole Final Programme can be found at:
http://orgs.dermis.net/content/e04scur/e03meetings/e775/e985/index_ger.html
or visit http://www.scur.org and click “Meetings” and the sublink: >Previous Meetings<
**ABSTRACTS**

**ORAL PRESENTATIONS**

**O 1**

Cell adhesion and apoptosis after γ-rays in human skin

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Skin is a target organ for crucial side-effects of routine radiotherapy. The pathophysiology of the cutaneous radiation reaction is for many aspects still unknown and the early effects on epidermal compartment remain to be fully elucidated. Organelloytic properties of normal human breast skin can help in defining the immediate radiation response otherwise difficult to study in human volunteers. Using this experimental model, we reported a significant inhibition of epidermal proliferation 24 hours after the exposure to a single dose of ionising rays (Donetti et al., 2005). In the present study we evaluated the early radiation effect on intercellular adhesion and apoptosis. Biologic fragments (n = 8) were obtained from cosmetic surgery of young healthy women, cultured epidermal side up in Transwell, and harvested 24 or 48 hours after exposure to a single 2 Gy dose of γ-rays. Samples were processed for transmission electron microscopy and immunofluorescence to investigate desmoglein (Dsg1) and p53 expression. Throughout the epidermal compartment, Dsg1 distribution pattern was similar in all non-irradiated and irradiated samples. Preliminary observations indicated that no desmosomal ultrastructural modifications occurred, suggesting that intercellular adhesion is not yet affected. At 24 hours, in both irradiated and non-irradiated samples p53 immunofluorescence was similarly detected in scattered positive keratinocytes in the spinous layer. At 48 hours, p53 was expressed starting from basal layer, with a slight p53 staining increase in irradiated samples. Our results can help in improving the knowledge of regulatory processes as a major prerequisite for radiobiological rationale effective interventions.

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**O 2**

Inducible activation of the hedgehog pathway by Gli-1 or Gli-2 expression in the human keratinocyte line HaCaT, growing in skin-organotypic coculture

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Transcription factors of the hedgehog (Hh) pathway like Gli-1 and Gli-2 are involved in basal cell carcinoma (BCC) formation (deriving mainly from hair follicles). To elucidate the role of Gli-1 and Gli-2, human keratinocytes (HaCaT-cells) were transfected with inducible vectors harboring either factor (Tet-on system). In conventional culture these HaCaT-variants over-expressed either Gli-1 or Gli-2 upon tetracycline (tet) treatment. In both tet-induced cultures proliferation increased and differentiation was repressed, abolishing the markers keratin 10 (K10) and filaggrin (immuno-fluorescence, IF). Mimicking skin physiology, cells were grown in three-dimensional skin-organotypic coculture (with dermal fibroblasts). Without Gli induction stratified, regularly differentiated epithelia developed (IF) like in normal HaCaT-controls. Upon Gli induction epithelial thickness was largely reduced initially, though cells, densely packed, increased in number (nuclear staining), while epidermal differentiation was blocked. Accordingly, K10 was largely and loricin completely absent, filaggrin appeared weak and irregularly distributed. Conversely, the 'simple epithelial-type' keratins K8/18 were strongly upregulated by both Gli-factors. Components of the basement membrane zone (nudrogens, laminin, integrin (b)4) revealed no visible differences (IF). With a pronounced delay (four weeks) some invasive growth (mainly Gli-2 mediated) into the 'dermal' part of cocultures was observed, suggesting crosstalk with the fibroblasts. This was underlined by matrix metalloproteinase 1 (MMP1) expression which revealed a complete shift of MMP1 mRNA (in situ hybridizations) from the basal HaCaT-cells to adjacent fibroblasts, individually presenting also myofibroblast markers. Collectively, epithelial differentiation is repressed by activation of the Hb pathway, resembling a BCC phenotype, and the enforced Gli-expression may provoke invasive growth in these 3D-cocultures.

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**O 3**

Immunohistochemical and ultrastructural studies in a patient with a Kindler syndrome-like disorder

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Kindler syndrome is an autosomal recessive genodermatosis characterized by trauma-induced acral skin blistering from infancy, photosensitivity, generalized poikiloderma, and diffuse cutaneous atrophy. Mutations in the KIND1 gene that encodes for the focal contact-associated protein kindlin-1 have been identified in several patients although the spectrum of clinical pathology within the cutaneous basement membrane zone.

To confirm by electron microscopic study. The patient received adjuvant local radiation therapy.

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**O 4**

Merkel cell carcinoma diagnosed by EM on formalin-fixed tissue

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A 56-year-old woman was referred to us for treatment of a skin tumor involving her right lower leg up to 2 years of history of tumor growth. A nodule on her right lower leg which grew from 2 cm in diameter to 3 cm × 3 cm × 1 cm. Biopsy of the lesion showed proliferation of small and round cells with numerous scattered mitoses, indicating malignancy. Patient otherwise was healthy, and neither physical examination nor systematic investigations including laboratory tests and CT scans of the body revealed any signs of lymphnode or distant metastasis. The lesion then was excised with a resection margin of 2 cm with the defect covered by skin graft. Pathologic examination of routinely stained sections revealed that the dermis was invaded by a tumoral proliferation made up of round, uniform cells with large, hyperchromatic nuclei with surgical margin negative. Immunohistochemically, tumor cells were positive for CD56, VS38c, Ks-67, AE1 and AE3. The diagnosis was confirmed by electron microscopic study. The patient received adjuvant local radiation therapy.

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O 5 Cutaneous Metatypical Carcinoma

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Metatypical carcinoma (MTC) is a controversial entity that presents itself as a microscopic pathway of both basal and squamous cell carcinoma. We recognized two types of histopathological patterns: a mixed and an intermediately one. The mixed type is composed of different cells: basaloid cells, which are larger, paler and more rounded of the cells of a classical basal cell carcinoma; squamousoid cells with copious eosinophilic cytoplasm; in some cases it is possible to find some areas composed of cells presenting an intermediate differentiation. The intermediately type is composed of nests and strands of cell maturing into larger and paler cells. As a distinctive feature, there is a large amount of spindle-cells. Peripheral palisading is often lost, and the stroma is prominent. The World Health Organization’s (WHO) histologic classification of skin tumours (Geneva, 1980) was the first to classify this neoplastic variant. MTC, which is typically located on the face, and often resembles clinical features of basal cell carcinoma (BCC) but tends to be more aggressive with the possibility of lymph node or distant metastasis.

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O 6 A recycling endosome marker Rab11 is associated with epidermal lamellar granules


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Epidermal lamellar granules (LG) are specialized organelles that transport and secrete various molecules, including lipids, proteins, protease inhibitors and structural proteins, thereby providing a protective barrier against the environment. Abnormalities in LG-related molecules result in severe skin diseases, but their transport mechanisms are poorly understood. We studied the distribution of Rab11, a common GTPase in recycling endosomes, in normal human epidermis. Confoocal laser scanning microscopy detected Rab11 immunoreactivity in differentiated epidermal keratinocytes. Staining was strong at the apical side of each cell, a pattern commonly seen in LG-associated molecules. Around the nuclei, Rab11 was colocalized with TGN46, a trans-Golgi network marker. Rab11 was also colocalized with known LG-molecules, namely LK1, cornemodulin, catherine D and glucosylceramides. Immunoelectron microscopy revealed that Rab11 was widely distributed along TGN and tubular-vesicular structures containing different LG molecules. The present results suggest that Rab11 plays a role in the intra- and intercellular recycling of LG-associated molecules. Based on the structural similarity between LGs and recycling endosomes in other cells and the present demonstration of strong Rab11 expression around LGs, we hypothesized that LGs are modified endosomes specifically developed in the epidermis.

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O 7 A genetic and ultrastructural study of Tietz syndrome

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Tietz syndrome is an extremely rare genetic disorder characterized by generalized hyperkeratosis and complete neural deafness. Only two families were reported so far and mutations in the microphthalmia-associated transcription factor (MITF) gene have been described in each family as a cause of the disease. A 24-year-old Japanese female, who was a product of non-consanguineous Japanese parents, was referred to our hospital for evaluation of generalized hyperkeratosis, and pigmentation flecks. She had a congenital hearing loss. Her skin was white and small pigment flecks were seen on sun-exposed area that began to develop at the age of three. Her hair was gray and her eyes were blue. Nystagmus and other ocular abnormalities were absent. A dopa reaction test using hair bulb was positive. A skin biopsy from the hypopigmented region revealed that melanocytes were present in normal density and distribution but adjacent basal keratinocytes contained little melanin. Electron microscopy of the melanocytes showed stage I to II melanocytes with irregularity in size and shapes. The direct sequencing of the MITF gene of the patient revealed heterozygous 3-bp in-frame deletion in exon 7 leading to a single amino acid deletion, delR217, which was identical mutation to that of a previously reported family. Our study indicates that, out of the two major roles of MITF, the development/maintenance of melanocytes and the regulation of melanin biosynthesis, the latter role may be more sensitive to the abnormality of MITF protein in Tietz syndrome.

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O 8 Melanoma cells circulating in peripheral blood

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Monitoring of melanoma cell levels in peripheral blood of melanoma patients is a promising method for haematogenous melanoma distribution. The method enables us to detect early metastasis and to better stratify candidates for adjuvant immunotherapy procedures. Inconsistent data on the sensitivity and clinical relevance of this method have been previously reported by several groups. In the present work the development of multimarker real-time RT-PCR for quantification of 5 melanoma markers could be shown. Following markers were detected: Melan-A, gp 100, MAGE-3, Mia and tyrosinase. In the described prospective study 65 patients with resected cutaneous melanoma stage IIB-III were screened. Samples of peripheral blood were collected every 3 months for the following 18 months and circulating melanoma cells were examined and compared with clinical staging methods. In 18 patients a relapse during the trial was observed and members of this subgroup showed different types of melanoma progression. In all of these patients statistically significant tumor marker elevation in the period from 0 to 9 months before the disease progression was noticed. MAGE-3 was better reflected by the structural similarity between LGs and recycling endosomes in other cells and the present demonstration of strong Rab11 expression around LGs, we hypothesized that LGs are modified endosomes specifically developed in the epidermis. Based on the structural similarity between LGs and recycling endosomes in other cells and the present demonstration of strong Rab11 expression around LGs, we hypothesized that LGs are modified endosomes specifically developed in the epidermis.

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O 9 Ultrastructural and molecular study in two patients with bullosous congenital ichthyosiform erythroderma

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We report two sporadic cases with bullosous congenital ichthyosiform erythroderma presenting different courses of the disease. First patient was a 16-months-old girl born in full term from non-consanguineous healthy parents who presented at the birth generalized blisters and erosions accompanied by Nikolsky’s sign. At the beginning of the third month of life the patient developed slight hyperkeratotic plaques on erythematous base localized on extensor surfaces of the forearms and calves and on the lower abdomen. While patient was 6-months-old she developed hyperkeratotic plaques and prominent palmoplantar keratoma (PPK) whereas blisters appeared occasionally. After 15 months of life blisters stopped to appear and hyperkeratotic scales dominated in the clinical picture. Second patient is a 24-month old girl born in full term from non-consanguineous healthy parents who presented at birth with disseminated blisters and erosions which ceased at the age of 16 months of life. At the beginning of third month of life the patient developed hyperkeratotic plaques on erythematous base localized on extensor surfaces of the forearms and on the lower abdomen. PPK has never been observed. Histopathology performed in both patients showed hyperkeratosis, acanthosis and the presence of numerous cells in the upper part of stratum spinosum with large keratohyaline granules, whereas electron microscopic study disclosed keratin clumps reflecting the disturbances in the keratin assembly process. Molecular analysis of the proband with PPK showed a mutation N188K in the exon 1 of KRT10 gene encoding keratin 10 resulting in an asparagine-to-lysine substitution, whereas in probands without PPK the mutation (g.984G>A) in the hot spot within the 1A domain of gene KRT10 encoding keratin 10 was found resulting in an arginine-to-histidine substitution. Our study confirms previous observations concerning strong association between mutations in the KRT10 gene and PPK in patients with bullosus congenital ichthyosiform erythroderma.

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O 10 Cellular retinol binding protein-1 expression in skin tumors

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Retinoids play a relevant role in growth and differentiation of epidermal cells. Cellular retinol binding protein-1 (CRBP-1) is a 15,000 Da cytosolic chaperone-like molecule that regulates the pre-nuclear phase of retinoid signaling. CRBP-1 expression also influences the uptake and subsequent esterification of retinol and its intracellular bioavailability. We analyzed the expression of CRBP-1 in normal and abnormal epidermal differentiation, actinic keratoses, squamous and basal cell carcinomas by immunohistochemistry and in situ hybridization. CRBP-1 immunodetection was low in the basal layer of normal epidermis, with increased expression in granular layer keratinocytes; CRBP-1 was completely absent in the stratum corneum. CRBP-1 expression was squamous and strongly expressed in skin adnexal structures. In phototic lesions, a hyperproliferative condition of the skin, an increased epidermal expression of CRBP-1 was also observed. In basal cell carcinomas, CRBP-1 expression was low expression of CRBP-1 was present in more differentiated squamous cell carcinomas, whereas it was reduced in less differentiated tumors. In general, CRBP-1 transcript in situ detection was well aligned with protein expression. In vitro, Western Blotting demonstrates that mouse epidermal, squamous cell lines maintain a variable CRBP-1 expression according to their phenotype. The treatment with all-trans retinoic acid induced an increase of CRBP-1 expression in human keratinocytes. Investigation of regulatory mechanisms of CRBP-1 expression may help to elucidate physiological changes occurring in keratinocytes during hyperproliferative and oncogenic processes. CRBP-1 immunodetection appears also as an adjuvant marker of keratinocyte differentiation.

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**O 11**

Melanocytes' differentiation: what happens to the stem cell?  
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Melanoblasts migrate in the skin and in other organs following a selective commitment; the activation of specific cytoplasmic and membrane receptors is mandatory for life, proliferation, migration and differentiation of these cells. Melanosome's organization is linked to a sophisticated intracellular secretory mechanism which gives the melanoblast/melanocyte a peculiar functional autonomy. The terminal differentiation of the melanocyte ends up in the skin and is affected by numerous cytokines, some with a phenotypic activity such as interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8) and some others including stem cell factor (SCF) and microphthalmia-associated transcription factor (MITF) with a high specificity for melanocytes differentiation. The specific terminal differentiation marker of melanocytes is the melanosome. In some pathological conditions such as metastatic melanoma, vitiligo and other hypomelanoses, the melanocyte looses its specific differentiation markers and this is due to a "differentiation process". Melanocytes, after many passages in vitro, progressively lose melanosomes and many markers of melanocyte lineage as HMB-45, Melan-A, S-100 etc. reproduce in vitro what happens in some pathological conditions. A better knowledge of what happens to the stem cell of the melanocyte lineage (an auto cycleing cell with the ability to produce differentiated cells) would have both a biological and therapeutic relevance.

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**O 12**

Cutaneous extranodal rhabdoid tumor: a case report  
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Extranodal rhabdoid tumors are rare neoplasms distinguished by a conspicuous morphological phenotype characterized by large epithelioid cells with abundant eosinophilic cytoplasm and paranuclear inclusions of intermediate filaments. Controversy exists as to whether the extranodal rhabdoid tumor is a distinct clinicopathologic entity or merely a shared phenotypic expression of histogenetically divergent tumors. Although originally described in tumors from pediatric kidneys, the rhabdoid phenotype has since been described in a variety of patient ages and extranodal sites. Additionally, the rhabdoid phenotype has emerged in cutaneous neoplasms, either as a pure extranodal rhabdoid tumor or a composite phenotype coupled with another malignancy. In the skin, isolated case reports and small series have demonstrated the rhabdoid phenotype in conjunction with melanomas, squamous cell carcinomas, epithelioid malignant peripheral nerve sheath tumors, and rhabdomyosarcomas. Regardless of the clinical setting, the rhabdoid phenotype is uniformly associated with aggressive biological behavior. We report the findings from a rare and very aggressive primary extranodal rhabdoid tumor of the skin.

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**O 13**

Specific cutaneous lesions in course of mantle cell lymphoma  
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Mantle cell lymphoma (MCL) is a type of non-Hodgkin's lymphomas. The neoplastic cells derive from B-lymphocytes in the mantle zone of lymphoid follicles. The disease most often occurs in older patients. The diagnosis is usually established at the stage of generalized lymphadenopathy, splenomegaly and dissemination to the bone marrow and the blood. Skin involvement occurs rarely. We report a case of a 63-year-old woman who was admitted to the Department of Dermatology because of massive infiltrated erythemas with hyperpigmentation on the face, on nasal conchae and the nape. The lesions appeared 6 months before admission. Additional findings were dysnes of nasal cavity, difficulties in speaking, increasing dyspnoea and nasal conchae. The lesions were positive for leucopenia, CD20+/CD23+/DR+/Lambda+/Kappa+96%, Lambda+/Kappa+93%, CD5+/CD10+/CD19+/CD22+ (93%), DR+/CD21+/CD24+/CD38+/CD79a+/CD20+/CD3+/CD5+/CD22+ (93%), Lambda+/CD19+/CD22+ (87%). Final diagnosis of MCL was established on the basis of immunophenotypic study of lymph node, where cells were found positive for CD20+, CD43+, CD23+, CD10−, C-myel, tfl- and MIB (in about 50% of the cells). CT scans of the neck and chest were made and revealed a tumor mass protruding into larynx fumen from aryepiglottic fold together with enlarged lymph node on both sides along cervical vessels and enlarged lymph nodes in the mediastinum. The patient was transferred on an oncology department where CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone) was administered. Improvement of general condition, both sides along cervical vessels and enlarged lymph nodes in the mediastinum. The patient was discharged on October 1998. In immunophenotypic studies of the bone marrow the neoplastic cells were positive for markers: CD5+, CD23+, DR+, CD21+, Lambda+, CD19+ and CD22+. The diagnosis of MCL was confirmed by sequence analysis of IgH and Kappa and Lambda and ISEM positive. Analysis of partial gene sequences showed presence of B. burgdorferi and ISEM positive. Analysis of partial gene sequences showed presence of B. burgdorferi and ISEM positive. Analysis of partial gene sequences showed presence of B. burgdorferi and ISEM positive. Analysis of partial gene sequences showed presence of B. burgdorferi and ISEM positive. Analysis of partial gene sequences showed presence of B. burgdorferi and ISEM positive.

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**O 14**

Immunocytochemical and genetic proof of Anaplasma, Borrelia and Bartonella spp. in cutaneous and heart manifestation of Lyme disease  
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The purpose of this work was to assess the presence of pathological agents of cutaneous and heart manifestation of Lyme disease. Rickettsiae are known to be present in tick vector and in reservoirs (rodents, wild and domestic mammals) individually or as co-infection of Borrelia burgdorferi s.s. The human monocytic and granulocytic ehrlichiosis (HME, HGA) was described recently in animals and humans in the USA and Europe. The group of patients with erythema migrans (EM) comprised 49 patients and with heart manifestation of Lyme carditis (LC) after EM 36 patients. The prohabs were examined clinically by specialists using histological, IGG, ECHO, RGF and other methods. The skin and heart biopsies were taken together with blood for electron microscopic (EM) and immunohistochemical detection of Borrelia burgdorferi s.s. Western blot (Biowestern, C.D.). Direct immunosorbent (ISEM) methods were performed from glutaeraldehyde (Sabatin) fixed material with monoclonal antibodies (Mabs) or with hyper-immune sera against mentioned pathogens. Observations were made with a JEOI 200CX electron microscope on negative stained or Epon embedded sections. Amplification of specific DNA and sequence analysis was performed with reca, gltA 16S rRNA primers by the Real time PCR with a LightCycler Roche and COX 2000 LX sequence. The Borrelia strains were isolated from 6 skin, 2 heart biopsies and 2 blood samples in BSK-II medium and strains were characterized phenotypically with Mabs using WB and ISEM. Isolation of Anaplasma, Ehrlichia and Bartonella spp. in HLA cells and Vero cells was not successful but 3 blood and 2 skin biopsies were PCR and ISEM positive. Analysis of partial gene sequences showed presence of B. atezelii, B. garinii and B. burgdorferi in the skin and heart samples. Anaplasma phagocytophilum (HGA) was proved in 1 heart and 2 skin biopsies with RT-PCR and amplices were sequenced. Anaplasma, Rickettsia sp. and Bartonella spp. were detected in PCR positive skin and heart samples also electron microscopically. Borrelia were around the vessels and inside collagen fibers far from inflammation. In contrast to Borrelia other pathogens were inside cell inflammation i.e., neutrophils, monocytes and macrophages. Co-infections with mentioned agents can directly affect inflammation and caused different clinical feature of borreliosis.

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A 9-year-old boy presented with a generalized cornification disorder consisting of dirty-brown adherent scales. The face was less severely involved, palms and soles showed accentuation of markings. Ear helices were thickened and deformed, and the external auditory canals were filled with cerumen and keratoses compromising hearing. Mental and physical development seemed not impaired. The parents and the older brother were unaffected. Histology of a skin biopsy showed pronounced orthohyperkeratosis and focal hypergranulosis without signs of epidermal-lytic hyperkeratosis. On ultrastructural examination, the markedly thickened stratum corneum was composed of up to 90 lamellae and contained polygonal clefts. On cryostat sections, epidermal transglutaminase activity was completely absent. Based on clinical, ultrastructural, and immunohistological data a provisional diagnosis of lamellar ichthyosis (LI) caused by transglutaminase-1-deficiency was made which could be confirmed by detection of two different mutations in the gene encoding transglutaminase-1 (TGM1). Reduction of hyperkeratoses was achieved by urea-containing preparations and baking soda baths. There are at least 6 different types of LI which can only be distinguished genetically but not clinically. Among them, LI with underlying transglutaminase-1-deficiency, as demonstrated in our patient, is the most common type accounting for about a third of cases.

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