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Conradi–Hünermann–Happle syndrome with abnormal lamellar granule contents

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Sir, Conradi–Hünermann–Happle syndrome (CHH) (X-linked dominant chondrodysplasia punctata type II, MIM 302960) is an X-linked dominant inherited disorder, characterized by linear ichthyosis, chondrodysplasia punctata, cataract and short stature.[1] The gene for this disease has been identified as EBP encoding the emopamil binding protein (EBP), located on the short arm of the X chromosome.[2,3] However, the exact pathomechanisms of how EBP defects cause the CHH disease phenotype have yet to be clarified. Ultrastructural features of CHH epidermis have thus far not been reported in patients whose EBP mutations have been identified. Here, we have ultrastructurally examined the epidermis of a patient with CHH carrying the EBP mutation p.Arg147His and have demonstrated abnormal contents of lamellar granules in the lesional granular keratinocyte layers.

A female newborn with skin scaling and shortened extremities was referred to us. The pregnancy had been uneventful except for excessive amniotic fluid, and the baby was born spontaneously at 37 weeks 4 days gestational age by normal vaginal delivery. The birth weight was 2696 g and the height at birth was 44·0 cm. No respiratory distress was observed at birth. The baby was the second child of nonconsanguineous, healthy parents. There was no other affected member in the family, including the proband's healthy elder brother. Frontal bossing, flat nasal bridge and shortened neck were noted at birth (Fig. 1). The right upper and lower extremities of the patient were shortened. Whole body X-ray examination revealed punctate calcification in the epiphyseal regions of the majority of long bones (Fig. 1), including shoulder joints, elbow joints, wrist joints, hip joints, knee joints and ankle joints, and the calcification was most severe on the right side of her body. Her height was below the third percentile at birth and during all the postnatal period. Her weight was also below the third percentile from 2 months of age, although it was between the 10th and the 25th percentile at birth. She had bilateral anterior polar cataracts, which were more severe on the right side. During the neonatal period, linear and whorled hyperkeratosis was seen on erythrodermic skin and the thick scales were more severe on the right side of her

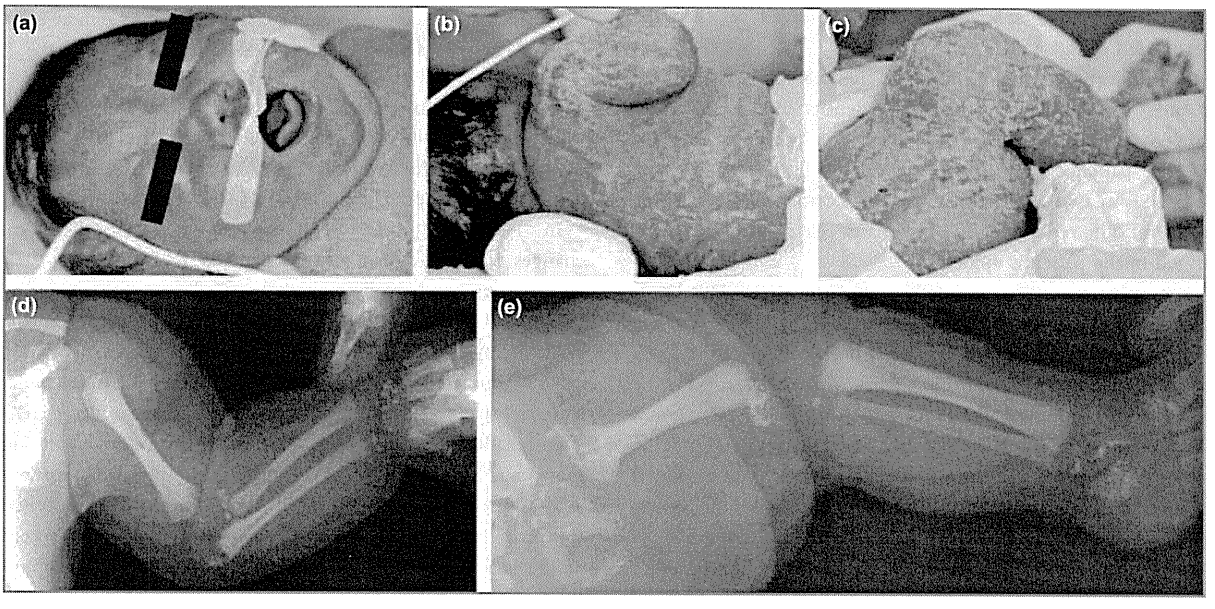

Fig 1. Clinical and X-ray appearance of the patient. (a) Frontal bossing and flat nasal bridge were seen on the face. (b) Circumscribed alopecia was noted on the scalp. (b, c) Linear and whorled hyperkeratosis was seen on a background of erythrodermic skin on the back (b) and over the thigh (c). (d, e) X-ray showed punctate calcification in the epiphyseal growth plate of the bones of the right arm (d) and in the right leg and hip (e).

body (Fig. 1). The entire body surface was erythematous and the extremities were oedematous. Circumscribed alopecia was more pronounced on the right side of the scalp. The skin eruptions cleared within several weeks, although slightly scaly skin remained over her whole body. Skin biopsy of a hyperkeratotic lesion on the trunk taken during the neonatal period revealed orthohyperkeratosis. A marked calcification in the stratum corneum was seen by van Kossa staining. From these clinical and histological features, a diagnosis of CHH was made in this case.

All four coding exons 2–5 of EBP were amplified using previously described polymerase chain reaction (PCR) primers.[4] Direct sequencing of the PCR products from the patient and her parents revealed that the patient was a heterozygote for a missense mutation p.Arg147His [G to A substitution at nucleotide position 440: arginine 147 (CGC) to histidine (CAC)], which was not found in her parents. This mutation was not found in 100 normal unrelated alleles (50 normal unrelated Japanese individuals) by direct sequencing analysis. Direct sequencing of all the coding exons and exon/intron borders of EBP failed to detect any other pathogenic mutations in the patient's DNA. The p.Arg147His is a known mutation reported in an aborted fetus affected with CHH.[3]

We performed ultrastructural observation of the patient's epidermal keratinocytes using ruthenium tetroxide postfixation. Lamellar granules with abnormal contents, lacking the normal lamellar structure, were seen in the granular layer keratinocytes in the patient's epidermis (Fig. 2). The lamellar granule contents were secreted into the intercellular space in the stratum corneum. Secreted lipid material trapped in the cytoplasm of corneocytes, corresponding to the membranous remnants reported by Emami *et al.*,[5] was distributed sparsely throughout the stratum corneum. In addition, irregularly dilated intercellular spaces were often observed between the keratinized cells.

We performed CD1a staining on the skin biopsy specimen in order to evaluate Langerhans cell density in the epidermis. Langerhans cell density was 8·8 cells/high power field (HPF) in the patient's epidermis and 4·2 cells/HPF and 16·0 cells/HPF in two age/gender-matched normal controls. Thus, no significant reduction of Langerhans cell density was confirmed in the patient's epidermis.

EBP mutations were reported to underlie CHH. EBP has a dual function: on the one hand it serves as a binding protein for the Ca^{2+} antagonist emopamil and thus is a high-affinity acceptor protein for several anti-ischaemic drugs,[6] and on the other hand it acts as a delta8–delta7 sterol isomerase.[7] It has been suggested that the skeletal manifestations in CHH may be caused by an accumulation of toxic sterol intermediates which interfere with the function of cholesterol-modified hedgehog proteins.[8] Furthermore, the molecular pathology of the ichthyotic phenotype in CHH can also be explained by EBP function in sterol biosynthesis pathways. EBP is a key enzyme involved in cholesterol biosynthesis[7] and dysfunction of EBP results in cholesterol deficiency and accumulation of cholesterol pathway products such as 8-dehydrocholesterol.[2,3] In a review of the pathophysiology of ichthyosis disease, Elias *et al.*[9] hypothesized that a deficiency of bulk cholesterol accumulation in keratinocyte membrane function may be a major factor contributing to the ichthyosis phenotype seen in CHH.

Fig 2. (a) Electron microscopy revealed irregularly dilated intercellular spaces (white arrows) between the keratinized cells and a small number of membranous remnants (black arrows) within the cytoplasm of keratinized cells. (b) In a granular layer cell, lamellar granules lacking lamellar structure, vacant or containing irregular sized vesicles (black arrows) were observed. Original magnification: (a) × 12 000, (b) × 60 000.

In 1984, Kolde and Happle[10] reported morphological changes in the lesional skin of patients with CHH. In their report, numerous small to medium-sized vacuoles measuring between 0·4 and 1·5 μm in diameter were observed in the granular layer keratinocytes, as seen in the present study. In 1994, vacuolated lamellar granules and a lack of intercellular lamellar structures were shown in an infant with CHH,[5] although the causative molecule or gene was not elucidated in those studies. In this report, for the first time, in a patient with CHH with a confirmed EBP mutation, we demonstrated abnormal lamellar granule contents in the granular layer cells in the lesional epidermis. The present ultrastructural findings suggest that EBP mutations and consequent cholesterol deficiency lead to defective lamellar granule contents, resulting in malformed intercellular lipid layers and the ichthyotic skin phenotype characteristic of patients with CHH.

Kolde and Happle[11] reported Langerhans cell degeneration and reduced density of Langerhans cells in the patients' epidermis and suggested that the ichthyotic phenotype of CHH is caused by Langerhans cell depletion. However, in the present study, no significant reduction of Langerhans cell density was observed in the patient's epidermis.

Kolde and Happle[10] reported that hair follicles showed signs of atrophy which was in an early stage in a 4-week-old baby, and was fully developed in a 14-year-old girl, although the other morphological abnormalities were similarly observed in both the baby and the 14-year-old girl. In the present study, no apparent atrophy of hair follicles was seen, probably because the skin biopsy sample was taken in the neonatal period.

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Acquired idiopathic generalized anhidrosis: possible pathogenic role of mast cells

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Sir, A 37-year-old man experienced anhidrosis of almost his entire body and cholinergic urticaria accompanied by severe heat intolerance for 5 months, which caused him to leave his job as an electrical engineer. A physical examination revealed no abnormalities except for slightly dry skin on his trunk and extremities. A neurological examination yielded no abnormal findings for his sensory system and sympathetic function. Laboratory tests, including blood counts, antinuclear antibody, anti-SS-A/Ro, anti-SS-B/La, total IgE and other biochemical profiles were normal except for a slightly elevated total bilirubin (1·2 mg dL^{-1}; normal 0·0–1·0 mg dL^{-1}).

Intradermal injection of 0·05 mL acetylcholine (100 μg mL^{-1}) produced no local sweating (Fig. 1). A thermoregulatory sweating test using the iodine–starch method showed almost generalized anhidrosis except for the axillary zones (Fig. 1). After 15 min of exercise on a treadmill, only 0·06 mL of sweat was collected from both forearms; pinpoint-sized weals characteristic of cholinergic urticaria were observed. In order to check his responsiveness to autologous sweat, autologous sterilized sweat (diluted 1 : 100) was injected intradermally, resulting in a negative response.[1] A skin biopsy specimen was taken from his right forearm where sweating did not occur. The eccrine glands and ducts were surrounded by infiltrates of CD3-positive lymphocytes and a considerable number of mast cells (0·88 mast cells per gland) that were metachromatically stained with toluidine blue (Fig. 2). Serial sections of the skin biopsy revealed focal hyperkeratosis at the acrosyringium and normal eccrine glands (Fig. 2).

The patient was diagnosed with acquired idiopathic generalized anhidrosis (AIGA) accompanied by cholinergic urticaria. Firstly, loratadine 10 mg daily was administered but this treatment was not effective for the cholinergic urticaria or anhidrosis. Next, methylprednisolone 1000 mg daily was

eradication treatment will probably give a huge advantage in terms of social health, especially in high-risk areas.

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CLINICAL OBSERVATIONS

Granulysin as a Marker for Early Diagnosis of the Stevens–Johnson Syndrome

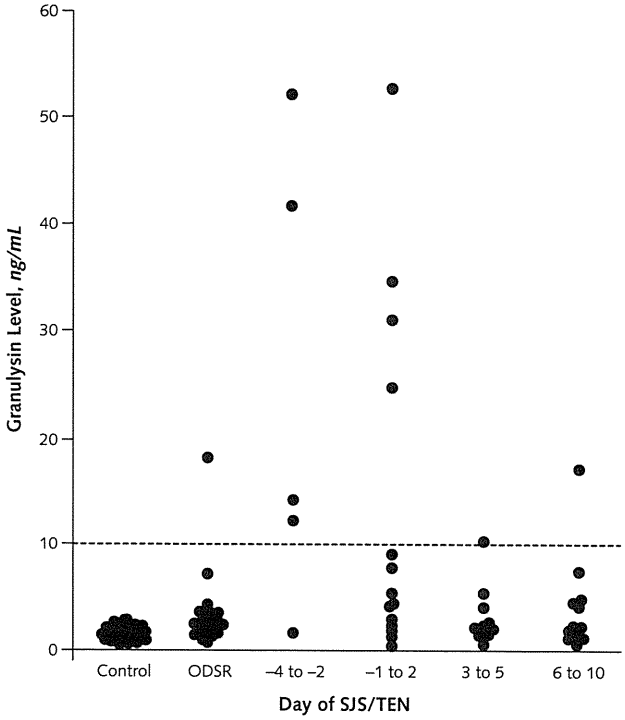
Background: The Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening adverse drug reactions characterized by massive epidermal necrosis. In the early stage, clinical presentations of SJS/TEN are very similar to those of ordinary drug-induced skin reactions (ODSRs); therefore, SJS/TEN is difficult to diagnose and the start of treatment is often delayed, resulting in high mortality rates. Other investigators (1) reported that granulysin is highly expressed in blisters of SJS/TEN and causes disseminated keratinocyte death. Because SJS/TEN progresses and spreads rapidly, the granulysin level should be increased in the serum of patients with active SJS/TEN if it is a key mediator of these diseases.

Objective: To determine whether serum granulysin levels are higher in patients with SJS/TEN than in healthy control participants or those with ODSRs.

Methods: We measured granulysin in the sera of 31 healthy control participants, 24 patients with ODSR, 13 patients with SJS, and 7 patients with TEN by using enzyme-linked immunosorbent assay (2). Disease onset in patients with SJS/TEN was defined as the day (day 1) on which the mucocutaneous or ocular lesion first eroded or ulcerated (3), and we collected sera from these patients from 4 days before to 10 days after ulceration. We used the Tukey–Kramer test to conduct multiple comparisons between groups.

Results: None of the 31 healthy control participants had a granulysin level greater than the upper limit of normal, which was 10 ng/mL (0% elevated; mean, 1.6 ng/mL [SD, 0.6]), and among 24 patients with ODSRs, only 1 patient had an elevated granulysin level (4.2% elevated; mean, 3.5 ng/mL [SD, 3.4]) (**Figure**). We obtained samples from 5 patients with SJS/TEN on day −4 to day −2, and we detected the highest granulysin concentrations (elevated in 80% of patients); mean, 24.8 ng/mL [SD, 21.2]). Granulysin levels were lower in the 14 samples collected on day −1 to day 2 (28.6% elevated; mean, 13.7 ng/mL [SD, 16.0]), and were even lower in the 10 samples collected from day 3 to day 5 (10.0% elevated; mean, 4.2 ng/mL [SD, 3.0]) and in the 13 samples collected from day 6 to day 10 (7.7% elevated; mean, 4.5 ng/mL [SD, 4.5]). When we compared granulysin levels from day −4 to day −2 among patients with SJS/TEN, patients with ODSRs, and healthy control participants, the differences were statistically significant ($P < 0.010$).

Figure. **Granulysin levels of healthy control participants, patients with ODSRs, and patients with SJS/TEN.**



ODSR = ordinary drug-induced skin reaction; SJS/TEN = Stevens–Johnson syndrome/toxic epidermal necrolysis.

Discussion: Granulysin is cytotoxic for tumor cells, transplant cells, bacteria, fungi, and parasites, in which it damages negatively charged cell membranes because of its positive charge (4). It plays an important role in the host defense against pathogens, and it induces apoptosis of target cells by using a mechanism involving caspases and other pathways (4). Its potency makes it a credible mediator of skin damage in patients with SJS/TEN. Adding to this credibility is a report (1) that granulysin is the most highly expressed cytotoxic molecule in the blisters of patients with SJS/TEN. We show that serum granulysin levels in 4 of 5 patients with SJS/TEN were elevated before skin detachment or mucosal lesions develop. Soluble Fas ligand (sFasL) shares some properties with granulysin: It contributes to keratinocyte death in SJS/TEN (3, 5), and levels are elevated in the sera of patients with SJS/TEN (3). Serum granulysin levels, however, are approximately 100 times higher than those of sFasL on day

−4 to day −2 (23.1 ng/mL [SD, 16.6] vs. 147.76 pg/mL [SD, 104.4]). Therefore, we believe it would be easier to develop bedside granulysin serum measurement, for example, by using immunochromatography, than it would be to develop a similar sFasL measurement. Monitoring serum granulysin might enable early diagnosis of SJS/TEN in patients with cutaneous adverse drug reactions that otherwise could not be distinguished from ODSRs.

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Localized Amyloidosis at the Site of Enfuvirtide Injection

Background: Enfuvirtide is the first of a new class of antiretroviral agents that block fusion of the viral particle with the host target cell. Its safety and antiviral activity have been demonstrated (1, 2). In clinical trials, injection site reactions occurred in 80% to 100% of patients (3). The most common signs and symptoms reported were induration in 94%, erythema in 91%, and subcutaneous nodules and cysts in 70% (4).

Objective: To describe a case of amyloidosis at the injection site of enfuvirtide.

Case Report: The patient was a man aged 47 years who had a history of sexual intercourse with men and extensive treatment for HIV with a triple-class viral resistance profile. He also had long-standing leg pain thought to be secondary to HIV neuropathy and no history of intravenous drug use. There was no history of opportunistic or chronic infections.

Because of a persistently elevated viral load, enfuvirtide by subcutaneous injection was added to his highly active antiretroviral treatment regimen for 41 months; enfuvirtide therapy was then stopped in February 2007 because of intolerable injection site reactions. While he was receiving enfuvirtide, his viral loads were completely suppressed. Eighteen months after enfuvirtide therapy was stopped, large, tender, indurated reactions with fragile epithelial surfaces persisted at all injection sites (**Figure**, *top*). These reactions bled extensively into the subcutaneous tissue with minor trauma (**Figure**, *bottom*). A lesion on the triceps was excised surgically, and the wound healed without complications. Pathologic examination showed extensive deposits of proteinaceous material with intense Congo red staining that was consistent with amyloid. A lesion on the opposite arm was resected and showed similar findings. The patient had a normal leukocyte count and normal hemoglobin, blood urea nitrogen, and creatinine levels and had no evidence of plasma cell dyscrasia and no history of organ dysfunction to suggest systemic amyloidosis.

Discussion: In 7 patients receiving enfuvirtide, biopsy of injection site reactions revealed an inflammatory response consistent with a localized hypersensitivity reaction (5), and other studies (3) have reported similar findings. Other reports (6) have described 3 histologic patterns: an acute urticaria- or vasculitis-like pattern with inflammation of the fat tissue, a subacute pattern with an initial dermal sclerosis, and a long-term scleroderma-like pattern.

In our patient, surgical excision of enfuvirtide injection site reactions revealed subcutaneous nodular amyloidosis. Localized

Figure. **Lesion in right triceps area (*top*) and periumbilical site with spontaneous intradermal and subcutaneous hemorrhage (*bottom*).**