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CLINICAL REPORT

Conradi-Hünermann-Happle Syndrome (X-linked Dominant Chondrodysplasia Punctata) Confirmed by Plasma Sterol and Mutation Analysis

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Conradi-Hünermann-Happle syndrome, or X-linked dominant chondrodysplasia punctata, is a rare genetic disorder characterized by skeletal dysplasia, stippled epiphyses, cataracts, transient ichthyosis and atrophic residua in a mosaic pattern. Mutations in the gene encoding the emopamil-binding protein have been identified as an underlying cause. A 5-year-old girl presented for evaluation of ill-defined patches of cicatricial alopecia. In addition, subtle follicular atrophoderma, esotropia, craniofacial asymmetry and short stature were noted. Her history revealed widespread scaly erythema and eye surgery for congenital cataract in the first months of life. Diagnosis of Conradi-Hünermann-Happle syndrome was confirmed by plasma sterol analysis showing markedly elevated levels of 8(9)-cholestenol and 8-dehydrocholesterol and by detection of a missense mutation (c.307G>A; p.E103K) in the emopamil-binding protein gene. We suggest that plasma sterol analysis is a reliable method of establishing the diagnosis of Conradi-Hünermann-Happle syndrome, even in patients with less striking phenotypical changes beyond infancy. ***Key words: Conradi-Hünermann-Happle syndrome; X-linked dominant chondrodysplasia punctata; emopamil-binding protein; cholesterol biosynthesis; plasma lipid analysis.***

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Chondrodysplasia punctata is a term used for a heterogeneous group of skeletal disorders that are characterized by abnormal foci of calcification at the epiphyseal plates, causing radiographic stippling (1). Among them, Conradi-Hünermann-Happle (CHH) syndrome, or chondrodysplasia punctata type II (CDPX2; MIM 302960), is a rare X-linked dominant entity, which was fully delineated by Happle (2) in the late 1970s.

The phenotype of CHH syndrome is of greatly variable severity, ranging from stillbirth or early lethal forms to mild, clinically almost undetectable, manifestations. The syndrome occurs almost exclusively in females and is characterized by skeletal abnormalities, cataracts and characteristic skin lesions. Cutaneous involvement typically starts as a severe congenital ichthyosis with adherent large scales following the lines of Blaschko on an erythrodermic background. Collodion baby may precede this typical appearance. After some months, the ichthyosiform lesions regress and follicular atrophoderma remains. These patchy or linear, atrophic lesions with follicular accentuation, often on a hypo- or hyper-pigmented base, are reminiscent of orange-peel skin (2). Mild ichthyosis may persist, particularly on the limbs. Patchy cicatricial alopecia with coarse, lustre-less hair often emerges on the scalp, and represents the most conspicuous constant cutaneous feature in many cases. Mild nail changes, such as platonychia or onychoschisis, may be present.

Abnormalities of the skeletal system are variable and include epiphyseal stippling of long bones and vertebrae, resulting in premature ossification and asymmetric shortening of the long bones, dystrophic calcification of cartilaginous structures, commonly the epiphyses of the long bones, dysmorphic facies with frontal bossing, malar hypoplasia and flat nasal bridge, and scoliosis (3). Interestingly, the calcifications typically disappear in the first year of life (4).

Two-thirds of patients have cataracts at birth, usually asymmetrical and often unilateral (5). Other eye abnormalities include microphthalmus, nystagmus, glaucoma and optic nerve atrophy.

Mutations in the gene encoding the emopamil-binding protein (*EBP*), which functions as a 3β-hydroxysteroid-Δ8-Δ7-isomerase in the final steps of cholesterol biosynthesis, have been identified as an underlying cause of CHH syndrome (6). This enzyme defect leads to accumulation of 8-dehydrocholesterol and 8(9)-cholestenol in the plasma (7), which can be determined by gas chromatography-mass spectrometry (GCMS). Both molecular genetic analysis and plasma sterol evaluation were used to establish the diagnosis of CHH syndrome in a sporadic female case of preschool age with scarring alopecia, in which the diagnosis had been missed in infancy.

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CASE REPORT

A 5-year-old Turkish girl presented with unusual persistent alopecia and an eye problem (esotropia of the left eye). Her parents were healthy and unrelated, and she had an older normal sister. The mother had had a previous miscarriage at 8–10 weeks of gestation, for which post-mortem information was not available. The patient was born spontaneously after an uneventful pregnancy at 36 weeks of gestation. At birth, thickened and diffusely reddened skin with large adherent scales in a patterned arrangement was noted. At age 3 months, erythroderma and scaling slowly disappeared. According to the mother, scaling of the scalp persisted for some months.

A congenital cataract of the left eye and a minor congenital sectorial cataract of the right eye (Fig. 1) were diagnosed soon after birth. The cataract of the left eye was removed surgically at age 6 weeks, and an artificial lens was implanted at age 3 years.

At her first visit to our department, the girl showed ill-defined patches of cicatricial alopecia with single twisted hairs. The remaining scalp hairs appeared sparse, fine and dry (Fig. 1B). Her eyebrows and eyelashes were also scanty. Examination of the skin revealed areas of slight hypopigmentation with atrophic streaks and follicular atrophoderma in Blaschko-linear arrangement on the trunk, shoulders and arms (Fig. 2A and B). Both alopecia and atrophoderma predominated on the left body site. The girl's face was slightly dysmorphic with frontal bossing, saddle nose and hypertelorism. Further skeletal abnormalities included short stature (corresponding to the 3rd percentile) and minor asymmetrical shortening of the right leg with slight genu valgum and pes planovalgus, which did not require treatment according to orthopaedic opinion. Mental and psychomotor development appeared normal. X-rays of the right foot showed no epiphyseal changes.

Analysis of plasma sterols was performed by GCMS using a method slightly modified from the procedure published elsewhere (7). In brief, ethylene diamine tetracetic acid plasma was hydrolysed with degassed 4% ethanolic potassium hydroxide. 5a-cholestane was added as an internal standard. The extraction was performed with n-hexane and the sample was derivatized with MSHFBA (N-methyl-N-trimethylsilylheptafluorobutyramide) to form the trimethylsilyl derivatives. For GCMS analysis the quadrupole mass spectrometer MSD 5972A (Agilent Technologies, Böblingen, Germany) was run in the selective ion monitoring mode.

The following characteristic mass fragments were used for quantification: m/z 217/357 (5a-cholestane, internal standard), m/z 329/368 (cholesterol), m/z 213/229 (8(9)-cholestenol), m/z 325/351 (8-DHC). In our patient 8(9)-cholestenol and 8-dehydrocholesterol were clearly elevated (1.19 mg/dl, normally not detectable, and 0.33 mg/dl, normal < 0.11 mg/dl, respectively). The cholesterol concentration was within the normal range (137 mg/dl). Unlike the patient, her parents and her sister showed no deviation of 8(9)-cholestenol and 8-dehydrocholesterol levels.

Following informed consent, genomic DNA was extracted from peripheral blood lymphocytes. The coding exons of the *EBP* gene were amplified using primers and polymerase chain reaction conditions, as described elsewhere (6, 8). Amplified exon sequences were analysed by genomic sequencing. The direct sequencing analysis revealed that the patient was heterozygous for a transition of one guanine at nucleotide position 307 to adenine (c.307G > A) in the *EBP* gene. The missense mutation, which exchanges an amino acid in EBP (p.E103K), was not found in the mother and the sister of the patient. An amplification-refractory mutation system (ARMS) test

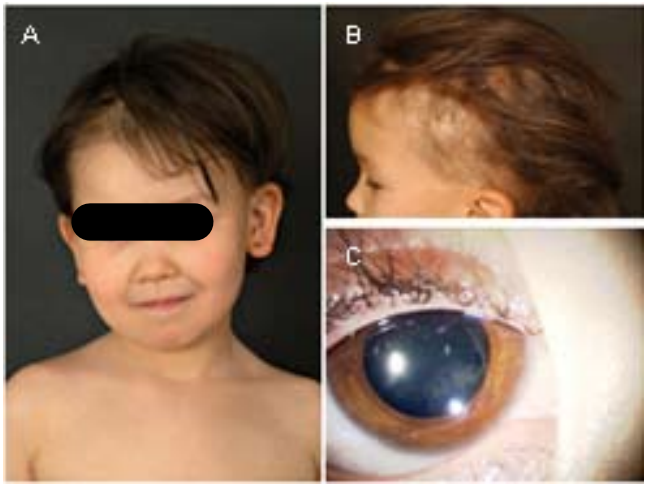

Fig. 1. (A) Slight craniofacial asymmetry, frontal bossing and esotropia of the left eye. (B) Ill-defined patches of cicatricial alopecia. (C) Sectorial cataract of the right eye.

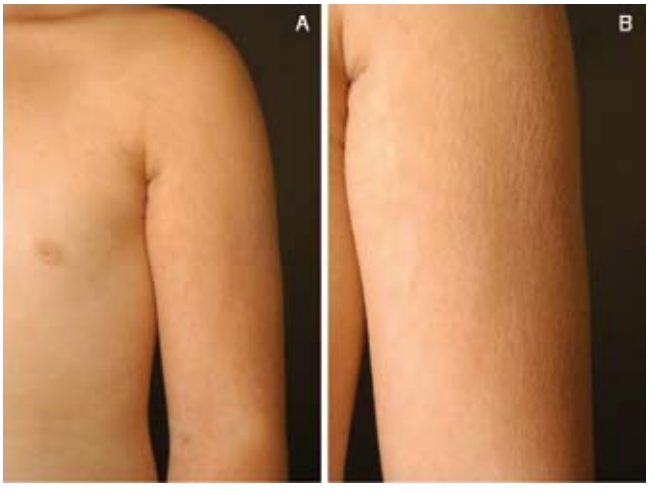

Fig. 2. (A) Discrete linear areas of hypopigmentation on the left side of the trunk and left upper arm. (B) Closer view showing linear atrophy of the skin.

was performed to confirm the mutation in the patient and to exclude the possibility that it represents a polymorphism, by studying 100 control individuals.

DISCUSSION

We present here the case of a 5-year-old girl with CHH syndrome confirmed by plasma sterol quantification and mutation analysis. The diagnosis was missed in infancy, at a time when erythroderma and peculiar scaling in a whorled or wavelike pattern usually facilitate identification of this particular congenital ichthyosis. Moreover, a biopsy of the newborn skin would probably have allowed detection of keratotic follicular plugs containing intracorneal dystrophic calcifications, which seem to be pathognomonic of CHH syndrome (9). Later on, histopathological features are no more specific, which is why we refrained from taking a biopsy in our patient.

The most common dermatological sign beyond infancy and the cause of presentation in our patient were patchy areas of cicatricial alopecia on the scalp. The term alopecia ichthyotica has been coined for scarring alopecia in conjunction with, or as a consequence of, different types of ichthyosis, among them CHH syndrome, autosomal recessive lamellar ichthyoses, ichthyotic neutral lipid storage disease Dorfman-Chanarin and keratitis-ichthyosis-deafness syndrome (10). The latter conditions could easily be ruled out because of differing associated features and complete regression of ichthyotic skin changes in our patient. The combination of patchy cicatricial alopecia with eye involvement including strabismus and minor skeletal changes in a female patient was also suggestive of incontinentia pigmenti. However, its typical course with vesicular and verrucous lesions preceding the development of linear hyper- and hypo-pigmented areas differed significantly from the history notified by the parents of our patient and available information. Scarring alopecia and aplasia cutis-like lesions are also found in focal dermal hypoplasia, which is another X-linked dominant trait accompanied by linear skin atrophy and associated eye anomalies. Moreover, very rare cases with unilateral distribution of cutaneous and skeletal lesions, but without eye involvement require differentiation of CHH syndrome from CHILD syndrome (11), another X-linked dominant condition characterized by congenital hemidysplasia, ichthyosiform naevus and limb defects. Hardly visible, hypopigmented linear bands with slightly depressed and dilated follicular openings in terms of follicular atrophoderma on the arms, a certain craniofacial asymmetry, short stature with minor difference in leg length, and the ophthalmological history of bilateral asymmetrical cataracts led us to assume CHH syndrome in the girl presented.

Linear, patchy and asymmetrical involvement of skin, bones and lenses in CHH syndrome is believed to reflect X-chromosomal mosaicism subject to X-inactivation (12). Analogous to incontinentia pigmenti, reduced viability of the mutant clone in the epidermis is likely to underlie the change of a highly active dermatosis at birth to atrophic residua in later life (2). As ichthyosiform erythroderma, epiphyseal stippling typically resolves within the first months of life and was no longer found in the radiographs performed in our patient. Skeletal and ophthalmological features were also distributed in an asymmetrical pattern, reflecting the mosaic nature of the condition. Unilateral, asymmetrical or sectorial cataracts, as in our case, are a clinical hallmark of CHH syndrome. Further eye anomalies are much rarer, and esotropia, as seen in the girl presented, has been described in only one previous patient, a hemizygous male with severe atypical phenotype (13).

CHH syndrome is due to mutations in the gene encoding the EBP, first described as a receptor for emopamil and other anti-ischaemic drugs (14), was identified simultaneously in mice and humans (6, 8). The affected gene has been mapped to Xp11.22–p11.23, spans 7.0 kb of genomic DNA and comprises 5 exons encoding a 1.0 kb mature transcript. To date, at least 46 different mutations have been described (15).

There is no clear correlation between mutations found in the *EBP* gene and the severity of the clinical phenotype (16). This may be due to differences in X-inactivation in various tissues of the same patient and/or to loss of the mutant clone by outgrowth of proficient clones (12). Severity of the clinical phenotype and expressivity of a particular mutation have been postulated to reflect the pattern and timing of X-inactivation at least as much as the mutation itself (17). Other authors claimed that *EBP* mutations producing truncated proteins result in more typical cases with complete phenotype than missense mutations in which the phenotype is not always complete (18). Our patient's phenotype, resulting from a missense mutation, showed a complete, but not severe, phenotype with skeletal, ocular and cutaneous lesions.

A striking clinical feature of CHH syndrome is strong intrafamilial variation, also demonstrating that the phenotypic effect of a given mutation cannot be predicted (19). Variation of expressivity of the same mutation within the same family is of paramount importance when providing genetic counselling to CHH syndrome families. Phenotypic differences may be attributed to the effect of the mutation on enzyme function and to different patterns of X-inactivation in each patient (12).

Gonadal mosaicism in a parent is the most likely explanation for the finding of healthy parents who have two affected children. Clinically unaffected parents or parents with very mild disease expression may suffer from somatic mosaicism. Therefore, the inability to detect a mutation in the parents of a sporadic case does not completely exclude the risk of recurrence. Concerning

our patient, gonadal mosaicism in the parents cannot be ruled out with certainty, although they exhibited neither minor disease symptoms nor alterations of 8-dehydrocholesterol and 8(9)-cholestenol levels (19). This argues in favour of a *de novo* mutation in the affected child.

CHH syndrome is usually a lethal factor for male embryos. The rare occurrence of surviving males may be explained by post-zygotic mutation, supernumerous X-chromosome(s), or less deleterious mutations in hemizygous individuals (20).

CHH syndrome belongs to a group of genetic disorders that are due to disturbances of cholesterol biosynthesis. Affected individuals have abnormally increased levels of 8-dehydrocholesterol and 8(9)-cholestenol. Accumulation of these metabolites results from a deficiency of EBP, a 3β-hydroxysteroid-Δ8-Δ7-isomerase, which is a major enzyme of cholesterol biosynthesis catalysing an intermediate step in the conversion of lanosterol to cholesterol (6). The exact mechanism by which the disrupted enzyme function leads to impaired morphogenesis in skin, eye and bone is unknown. However, there is now evidence that sterol depletion, as in cholesterol biosynthesis disorders, such as Smith-Lemli-Opitz syndrome, CHILD syndrome and Greenberg dysplasia (21, 22), leads to a defective response to Hedgehog proteins. These secreted proteins act as morphogens in the regulation of embryonic patterning (23) and are responsible for the correct orientation of limbs, cartilage formation, cell migration around the notochord to form the primordial cartilage vertebral bodies, and enchondral growth (22).

Plasma sterol analysis is a highly specific and sensitive diagnostic tool in patients with suspected CHH syndrome. Accumulation of 8-dehydrocholesterol and 8(9)-cholestenol has been found in almost all patients with CHH syndrome (16). We therefore suggest sterol studies are performed before mutation analysis is considered to confirm the diagnosis. However, the extent of the biochemical alterations in the serum neither reflects nor predicts the clinical phenotype or genotype (24).

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