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LETTER TO THE EDITOR

High 8-dehydrocholesterol level in a typical case of Conradi—Hunermann—Happle syndrome with a novel H76Y missense mutation

KEYWORDS
Ichthyosis; Congenital genodermatosis; Cholesterol

The Conradi—Hunermann—Happle (CHH) syndrome (X-linked dominant chondrodysplasia punctata type II, MIM 302960) is an X-linked dominant disorder, characterized by segmental ichthyosis, chondrodysplasia punctata, unilateral cataracts, and short stature. The ichthyosis is arranged in whorls on the torso and in a linear pattern on the extremities, and follows the lines of Blaschko. The ichthyosis is often associated with erythema which fades away during the first several months of life. Skin lesions on the scalp usually result in scarring alopecia. Follicular atrophoderma is frequently noted as part of this condition. Facial dysmorphism, such as frontal bossing and flat nasal bridge is also a typical feature. The punctate calcification in the epiphyseal region leads to asymmetric development of the long bones, and congenital hip dislocation. The gene for this disorder has been identified as that encoding emopamil binding protein (EBP), located on the short arm of the X chromosome, Xp11.22—p11.23, and its molecular genetics have been clarified [1,2]. To date, 17 missense mutations, 18 nonsense mutations, 3 splice site mutations, 12 small deletions, and 5 small insertions have been reported in this gene [3]. Here, we describe a Japanese case of CHH syndrome with a novel missense *p.His76Tyr* mutation in *EBP*. Biochemical analysis of the patient's serum cholesterol also demonstrated increased level of 8-dehydrocholesterol.

A 1-day-old girl was sent to our institution for asymmetric limb shortening and hyperkeratotic scaling following the lines of Blaschko (Fig. 1a). Punctate calcification was found in the left shoulder joint, transverse pedicles of the vertebral bodies, hip joints, knee joints, and ankle joints (Fig. 1b). Localized ichthyosis was also present on the scalp, leading to partial alopecia (Fig. 1c). The circumference of the head was 34 cm, which is within normal range (30—35 cm). Frontal bossing and flat nasal root was mildly present (Fig. 1c). Transient tachypnea developed soon after birth, though respiratory status improved spontaneously. Ophthalmological examination was negative for cataract. General condition was fine, except for the dermatological manifestations. All four limbs were reduced in length and the left arm appeared much shorter than the right one.

Genomic DNA was extracted from peripheral leukocytes by the standard technique [4]. The four coding exons 2—5 were PCR-amplified using the intronic primers described elsewhere [5]. PCR products were gel-purified, and direct-sequenced from both sense and anti-sense primers with an Applied Biosystems 310 automatic sequencing machine. The mutation detected was re-sequenced by an independent repeated PCR amplification from the genomic DNA. To confirm that the mutation was not a polymorphism, we performed SSCP analysis [6] with a new primer pair: 5′-TTGCAGTGTGTGGGTTCAAT-3′/5′-GGATTATAAGCGTGAGCCAC-3′.

This study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine. Sterol concentrations were measured by high-performance liquid chromatography (HPLC). LC-9A HPLC system (Shimadzu corporation, Japan) and ODS-HG-3 column (2 mm i.d. × 25 cm, Nomura chemical Co. Ltd., Japan) was used. HPLC eluent was a mixture of acetonitrile, water and tetrahydrofuran (95:2:3). UV chromophore was combined with sterol for UV-HPLC detection. The details of the procedure used for analysis are now in preparation for publication (by T. Kasama et al.).

We found a novel heterozygous *p.His76Tyr* missense mutation in the *EBP* gene in a clinically typical Japanese case of CHH syndrome (Fig. 1d). The mutation was clearly discriminated by SSCP analysis,

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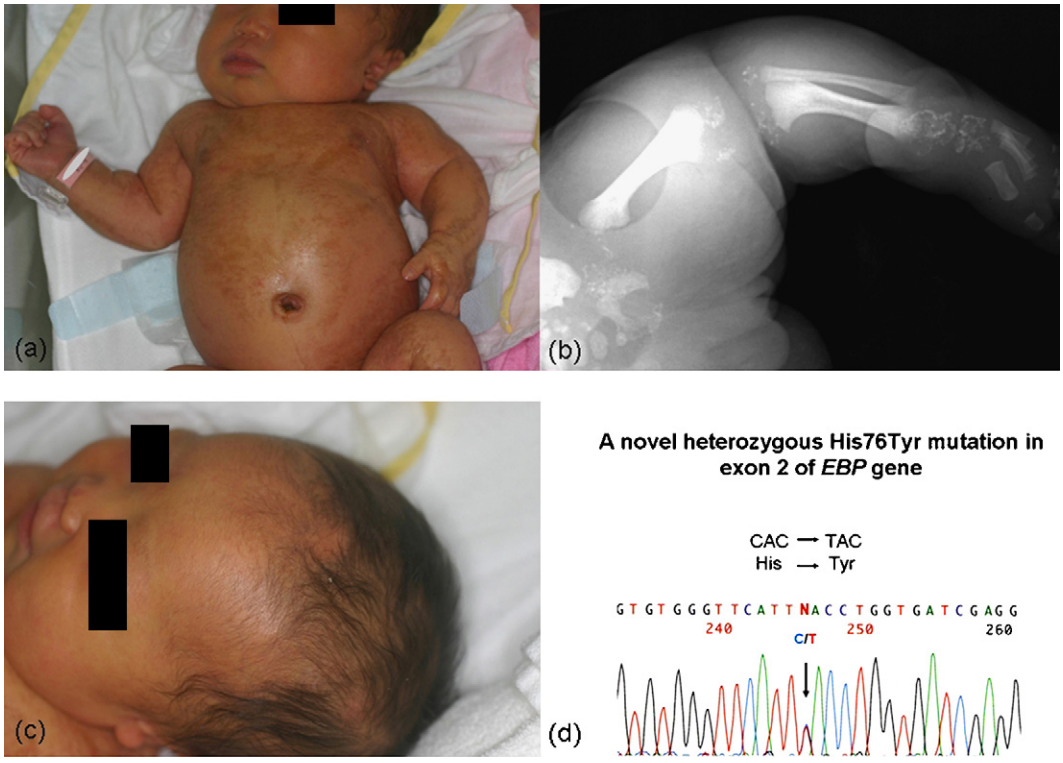

Fig. 1 (a) Generalized linear ichthyosis following the lines of Blaschko. (b) Punctate calcifications of the hip, right knee, and right ankle joint. (c) Partial alopecia on the scalp. (d) Heterozygous p.His76Tyr missense mutation was demonstrated by direct-sequencing.

in which no aberrant bands were observed in 55 healthy controls. This mutation was not listed in the JSNP nor dbSNP databases. The histidine76 is conserved among *Homo sapiens* (human), *Cavia porcellus* (guinea pig), *Mus musculus* (mouse), and *Arabidopsis thaliana* (arabidopsis) [7].

It has been reported that, in CHH, the serum level of 8-dehydrocholesterol is elevated [9]. In agreement with this, we found a markedly elevated 8-dehydrocholesterol (10.9 mg/L) in the patient's serum in our HPLC analysis. Usually no 8-dehydorochelesterol is found in normal subjects. 7-Dehydrocholesterol, which has been shown to be elevated in Smith—Lemli—Opitz syndrome, was not detected in this patient. The presence of 8-hydrocholesterol in our patient further supports the diagnosis of CHH.

Moebius et al. extensively examined the functional amino acids of the EBP protein. Numerous mutant *EBP* expression vectors were developed by site-directed *in vitro* mutagenesis [7]. Delta8—delta7 sterol isomerase activities were assayed by expression of these mutant EBP proteins in the sterol delta8—delta7 isomerization-deficient *erg*2—3 yeast strain. This study demonstrated that H76A, H76W, and H76N mutants exhibited less than 10% of wild-type catalytic activity. Systematic screening in a mutagenesis study also revealed that H76, E80, E122, T125, N193, and W196 were essential for enzymatic activity (the numbering of amino acid residues in Moebius' paper corresponded to the guinea pig EBP sequence, in which one amino acid [alanine] is inserted in the N-terminal region). These amino acids are located in the cytoplasmic halves of the transmembrane segments 2—5 and are predicted to face the catalytic cleft (Fig. 2). All six of these amino acids are conserved among the above four species. Notably, E80K [2] and W196S [8] missense mutations have been reported to cause CHH syndrome. The H76Y mutation is thus very likely to be pathological, and the causative mutation in the present case of CHH syndrome.

Although numerous mutations have been reported to date, no apparent genotype—phenotype relationships have been observed for CHH. Has et al. examined 11 families with CHH regarding severity of phenotype and 8-dehydrocholesterol level, and their relationships with genotype [9], but found no genotype—phenotype relationship. The 8-dehydrocholesterol level in the present patient (10.9 mg/L) was higher than those in any individuals of the 11 families (13 cases) studied by Has et al. [9]. At present, it is unclear whether 8-dehydrocholesterol level is correlated with severity of disease. Accumulation of cases