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Two novel frameshift mutations of the *EBP* gene in two unrelated Thai girls with Conradi–Hünermann–Happle syndrome

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Summary

Conradi–Hünermann–Happle syndrome, also known as X-linked dominant chondrodysplasia punctata (CDPX2), is characterized by skeletal abnormalities, cutaneous anomalies and cataracts. CDPX2 is caused by mutations in the *emopamil-binding protein* (*EBP*). We report two unrelated Thai female patients with clinically typical CDPX2, in which we discovered two novel and *de novo* frameshift mutations: 506–507delAG and 540–541delCC. This study demonstrates that *EBP* is the gene responsible for CDPX2 across different populations and extends the total number of confirmed mutations to 55.

Report

Conradi–Hünermann–Happle syndrome (MIM 302960), also known as X-linked dominant chondrodysplasia punctata (CDPX2), is characterized by skeletal abnormalities, cutaneous anomalies and cataracts. Skeletal abnormalities include craniofacial defects and epiphyseal stippling most noticeable around the vertebral column, pelvis and long bones resulting in short stature, kyphoscoliosis, and asymmetric rhizomesomelic shortness of the limbs. Cutaneous anomalies include coarse lusterless hair, alopecia, atrophic and pigmented lesions, striated palmoplantar hyperkeratosis, follicular atrophoderma, and ichthyosis.[1] CDPX2 is caused by mutations in the *emopamil-binding protein (EBP)* gene encoding 3β-hydroxysteroid-ρ8-ρ7-isomerase[2] and 53 mutations have been described.[3–8]

Here we report two Thai females with CDPX2 in whom we discovered novel mutations. There was no history of other family members being affected with the syndrome, consanguinity, excess miscarriages, or male stillbirths in either family.

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Patient 1 was a 2-week-old Thai girl born at term to a G1P0 mother. After birth, she was found to have erythematous ichthyosis of segmental distribution. Radiographs of her long bones and spine showed punctate calcifications of the epiphyseal regions (Fig. 1a).

Patient 2 was a Thai girl born at 38 weeks' gestation with a birth weight of 1800 g to a 24-year-old G2P1 mother and a 30-year-old unrelated father. The pregnancy and labour were unremarkable. According to her mother's report, at birth she had sparse hair and linear scaly skin symptoms on both legs, but CDPX2 was not diagnosed at that time. She had generally been healthy. She first presented to us at the age of 11 years, when she measured 122.5 cm (− 2.5 SD), weighed 26 kg (−1 SD), and had a head circumference of 51 cm (−1 SD). She had sparse and coarse hair, a 1 × 1-cm scarring alopecia of the scalp, linear and depigmented ichthyosis on extremities and trunk, asymmetric shortness of limbs with her left upper extremity being 3 cm longer than the right and her right lower extremity being 6.5 cm longer than the left (Fig. 1b), pes planus, and severe kyphoscoliosis (as seen in Fig. 1c). No signs of epiphyseal changes in radiographs could be seen at the time of presentation. Ophthalmic evaluation showed cortical cataracts bilaterally. Her IQ was 67.

After informed consent had been obtained, peripheral blood (3 mL) was obtained from the patients and their parents and DNA was extracted by standard methods.

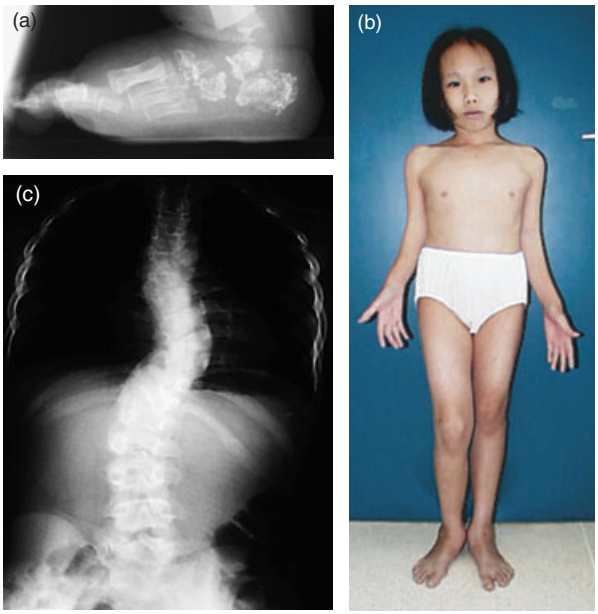

Figure 1 (a) Radiograph of patient 1 showed epiphyseal stippling. (b) Patient 2 had sparse hair, linear and depigmented ichthyosis on extremities and trunk, and asymmetric shortness of limbs. (c) Radiograph of patient 2 showed severe kyphoscoliosis.

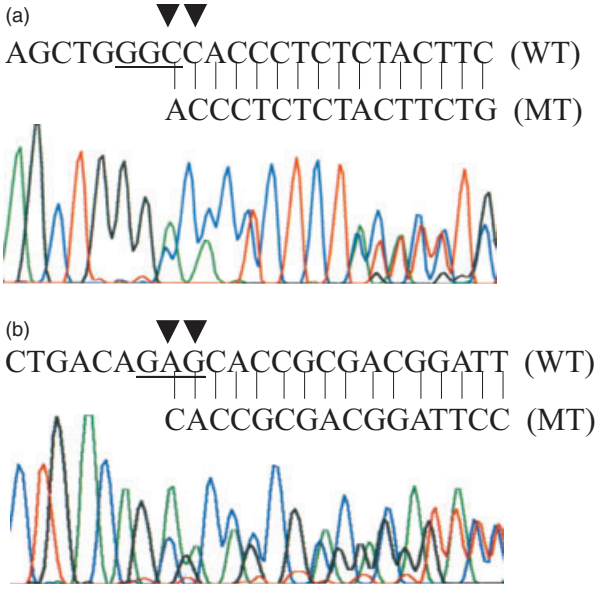

Figure 2 The sense sequence electropherograms of patient 1 (a) and patient 2 (b) showed the deletion of the two nucleotides (CC in patient 1, and AG in patient 2) (arrowheads). WT denotes wild-type sequence and MT stands for mutant. The first codons in wild-type sequences changed by the mutations are underlined. Patient 1 shows a heterozygous CC deletion resulting in a silent mutation of codon 180 (both GGC and GGA encode the same amino acid, glycine), subsequent changes of 24 amino acids and truncation at amino acid 204. Patient 2 shows a heterozygous AG deletion resulting in a conversion of a glutamate residue (GAG) at codon 169 to alanine (GCA), subsequent changes of 35 amino acids and truncation at amino acid 204.

Exons 2–4 and a part of exon 5, which contain the entire coding region of *EBP*, were amplified by PCR using primers (sense and antisense) for exon 2, 5′-TCGG TCCATTTACATTTCTC-3′ and 5′-CAAATCCCATCCCAC AGC-3′; exons 3 and 4, 5′-TGTGTGTTCCTTTCACTGCC-3′ and 5′-GGATACATCTGTGTCTGTGG-3′; exon 5, 5′-CCT CACTGGGGCTTCTCC-3′ and 5′-CTTCTGGCAGGCAGAG AGC-3′. The PCR products were treated with ExoSAP-IT (USP Co.), according to the manufacturer's recommendations, and sent for direct sequencing at the Macrogen Inc., Seoul, Korea.

Direct sequencing analysis of the PCR products revealed that patient 1 was heterozygous for a deletion of two cytosines at nucleotide positions 540–541 (540–541delCC) in exon 5 of *EBP* (Fig. 2a). The mutation is expected to result in subsequent changes of 24 amino acids and truncation at amino acid 204 because of a frameshift. The deletion was not found in her parents.

Patient 2 was found to be a heterozygote for deletion of nucleotides A and G at positions 506–507 (506–507delAG) in exon 5 of *EBP* (Fig. 2b). The mutation is expected to result in subsequent changes of 35 amino acids and truncation at amino acid 204 because of a frameshift. The deletion was not found in her mother. Her father was not available for the study.

We identified two unrelated Thai patients with a clinical diagnosis of CDPX2. Patient 1 had cutaneous and radiological features of CDPX2. Patient 2 exhibited complete phenotypes of the syndrome including cutaneous, skeletal and ophthalmic features. The clinical diagnosis of CDPX2 could be supported by sterol profiling or mutation analysis. However, sterol profiling can occasionally be misleading.[7] Mutation analysis showed that both of our two patients had frameshift mutations in exon 5 of *EBP*, expected to result in truncated nonfunctional polypeptide, confirming the clinical diagnosis of CDPX2. These mutations have not been reported previously.[3–8]

The nucleotides CC at positions 540–541 deleted in patient 1 are flanked by CT repeats three nucleotides away in both directions (535–550CTNNNCCNNNC TCTCT; CC are the deleted nucleotides). The nucleotides AG at positions 506–507 deleted in patient 2 are parts of repetitive sequences, 504–507AGAG. These findings support a previous observation that short (less than 20 bp) gene deletions causing human diseases have an endogenous sequence-directed mechanism of mutagenesis.[9]

The phenotype in female patients with CDPX2 ranges from mildly affected individuals diagnosed in adulthood to severely affected resulting in stillbirth. Correspondingly, mutations in the *EBP* gene vary from missense mutations with normal sterol profiles to nonsense or frameshift mutations with markedly abnormal sterol profiles.[7] Patient 1, a newborn, was too young to classify her clinical severity. Patient 2 had complete clinical features resulting from a frameshift mutation predicted to be a null allele. This observation supports previous suggestions that *EBP* mutations producing truncated proteins result in typical CDPX2 with complete phenotype including skeletal, ocular and cutaneous lesions, whereas the phenotypes resulting from missense mutations are not always complete for CDPX2.[10] Nonetheless, other studies showed that there was no clear evidence of a correlation between the molecular defects, levels of sterol intermediates, and the severity of clinical phenotypes in affected females.[2,5] This observation was strengthened by reports of intrafamilial variation in CDPX2.[1] The lack of a direct relationship between genotype and phenotype are likely to be due to lyonization, an important factor determining phenotypic severity of X-linked disorders. The *EBP* gene is presumed to be subject to X inactivation because of the mosaic phenotype of heterozygous females, such as the skin involvement, and because the gene is located in Xp11.22-p11.23 where many of genes undergo inactivation.[2]

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