A Novel Mutation of the *EBP* Gene Causes Conradi-Hunermann Syndrome

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Abstract A girl was born with rhizomelic limb shortening, narrow chest, generalised ichthyosis, and bilateral cataract. Skeletal X-ray showed wide spread stippled calcifications. Sterol metabolite analysis revealed elevation of 8-dehydrocholesterol and cholest-8(9)-en-3β-ol, consistent with Conradi-Hunermann syndrome, an X-linked dominant cholesterol biosynthesis disorder. Analysis of the *EBP* gene found a novel, heterozygous, 3 bp deletion in exon 2.

Key words Chondrodysplasia punctata; Conradi-Hunermann syndrome; *EBP* gene; Mutation

Introduction

Chondrodysplasia punctata is a heterogeneous group of disorders that share the common feature of punctate, or stippled, calcifications on skeletal X-ray. Collectively these disorders are very rare. The relatively common types are rhizomelic chondrodysplasia punctata type 1 (RCDP1; OMIM#215100), an autosomal recessive disorder, and the X-linked dominant type (CDPX2; OMIM#302960) also known as Conradi-Hunermann syndrome. CDPX2 is a cholesterol biosynthesis disorder. Cholesterol itself is an essential cellular substance. It is an important component of cell membranes and lipid rafts, and is the common precursor molecule for the synthesis of steroid hormones, bile acids and oxysterols. Cholesterol itself is synthesised in a series of about 30 enzymatic reactions starting from acetate. Some of the intermediary metabolites along this biosynthetic pathway are also intimately linked to a host of important cellular functions and signaling pathways.[1] The defective enzyme in CDPX2 is sterol Δ^8-Δ^7 isomerase. The gene, *EBP* (OMIM#300205), was mapped to chromosome Xp11.22-p11.23 and was identified in 1999.[2-4] Males carrying the defective gene usually do not survive to term; while females survive but manifest variable degree of facial dysmorphism, limb shortening, short stature, kyphoscoliosis, stippled calcifications on skeletal radiographs, ichthyosis, patchy alopecia, and cataracts. The limb shortening is usually rhizomelic and asymmetrical, and the ichthyosis follows the lines of Blaschko. This pattern of skeletal and skin involvement is the result of random X-inactivation in females. Intelligence is usually normal. Here we describe the clinical, biochemical and molecular findings in a girl with CDPX2.

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Received July 11, 2006

Case Report

Patient

TKS, a baby girl, was the only child of a non-consanguineous Chinese couple. The mother did not have history of abortions. There was also no family history of skeletal dysplasia, and the parents themselves had normal stature. Antenatal ultrasonography at 7 months' gestation already revealed short limbs and bowed legs.

She was born at maturity of 37+4 weeks by vaginal delivery with a birth weight of 2.53 kg. She was noted to have short neck, narrow chest, and rhizomelic shortening of the upper and lower limbs with asymmetrical involvement (Figure 1). Besides, she had generalised ichthyosis that appeared patchy and followed the lines of Blaschko. Other dysmorphic features were noted, including low-set and small ears, flat nasal bridge, right single palmar crease, and index finger camptodactyly. Ophthalmological examination also revealed bilateral cataract.

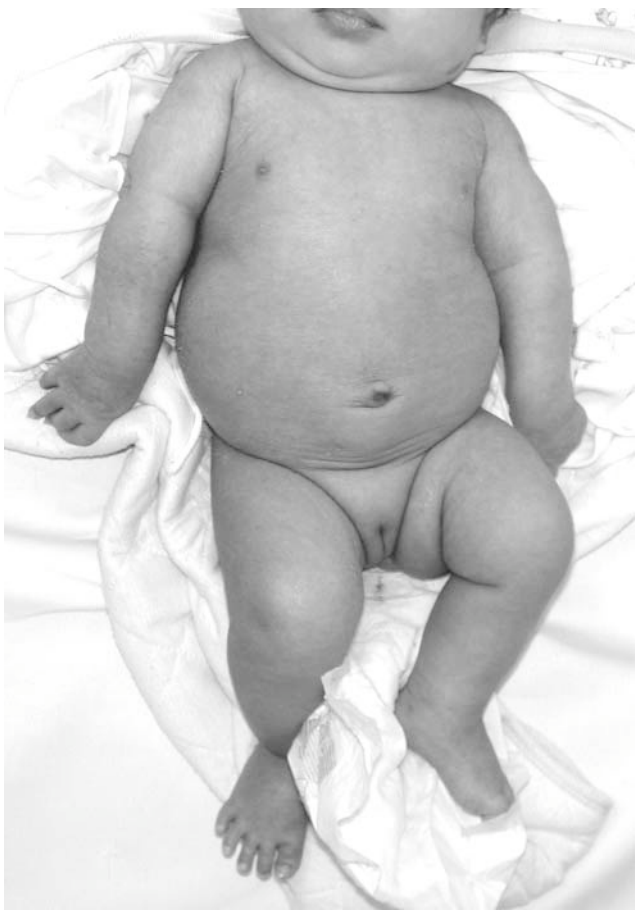

Figure 1 Clinical photo taken at the age of 2 months showed short neck, and predominantly rhizomelic shortening of the upper and lower limbs. Note left femur more severely affected than the right. Ichthyotic skin lesions were still present, but not as conspicuous as in the neonatal period and thus not clearly shown in this picture.

Skeletal survey by X-ray showed stippled calcifications at the hips, knees, ankles, shoulders, elbows, wrists, sternum, spine, and the tracheal and laryngeal regions (Figure 2). There is absence of coronal cleft in the lateral view of the spine.

Growth parameters at 2 months were: head circumference 36 cm (0.5 cm <3rd centile), body weight 3.87 kg (0.1 kg <3rd centile), and supine length 48.6 cm (4.5 cm <3rd centile). At 13 months, her head circumference was 44.5 cm (10-25th centile) and height was 63.6 cm (6.5 cm <3rd centile). Her development was satisfactory.

The clinical and radiological features were compatible with CDPX2.

Sterol Metabolite Analysis

The plasma specimen was hydrolyzed with ethanolic KOH for 1 hour at 80°C. The compounds were then extracted with hexane, followed by evaporation to dryness under nitrogen. The sterols were derivatized to generate trimethylsilyl ethers and injected into a capillary column. 8-dehydrocholesterol and cholest-8(9)-en-3β-ol were identified by electron impact gas chromatography-mass spectrometry (GC-MS) in full scan mode and quantified by selective ion monitoring. The analysis revealed elevation of 8-dehydrocholesterol and cholest-8(9)-en-3β-ol (Table 1).

EBP Gene Analysis

Genomic DNA was extracted from peripheral blood specimens using QIAamp DNA mini kit (Qiagen, Germany). The five exons and their intron-exon boundaries of the *EBP* gene were analysed by polymerase chain reaction (PCR) and direct sequencing. The PCR conditions and primer sequences were as described by Braverman et al (1999), except that the primer sequences for exon 2 are different (forward: 5'- TTT CTA TTT GTC CAG GTT TTT CTG T -3'; reverse: 5'- TCA GGA CTC ACA GAG TTG AGA TAA -3'). The reference sequence is the human *EPB* mRNA sequence (GeneBank accession number Z37986).

A de novo, heterozygous c.108_c.110delCTC in-frame deletion was detected in exon 2. The protein product was predicted to have deletion of the 37th amino acid, serine, in the first transmembrane domain. Besides, a heterozygous, maternally inherited c.15G>T nucleotide change was found in exon 2. This is a known single

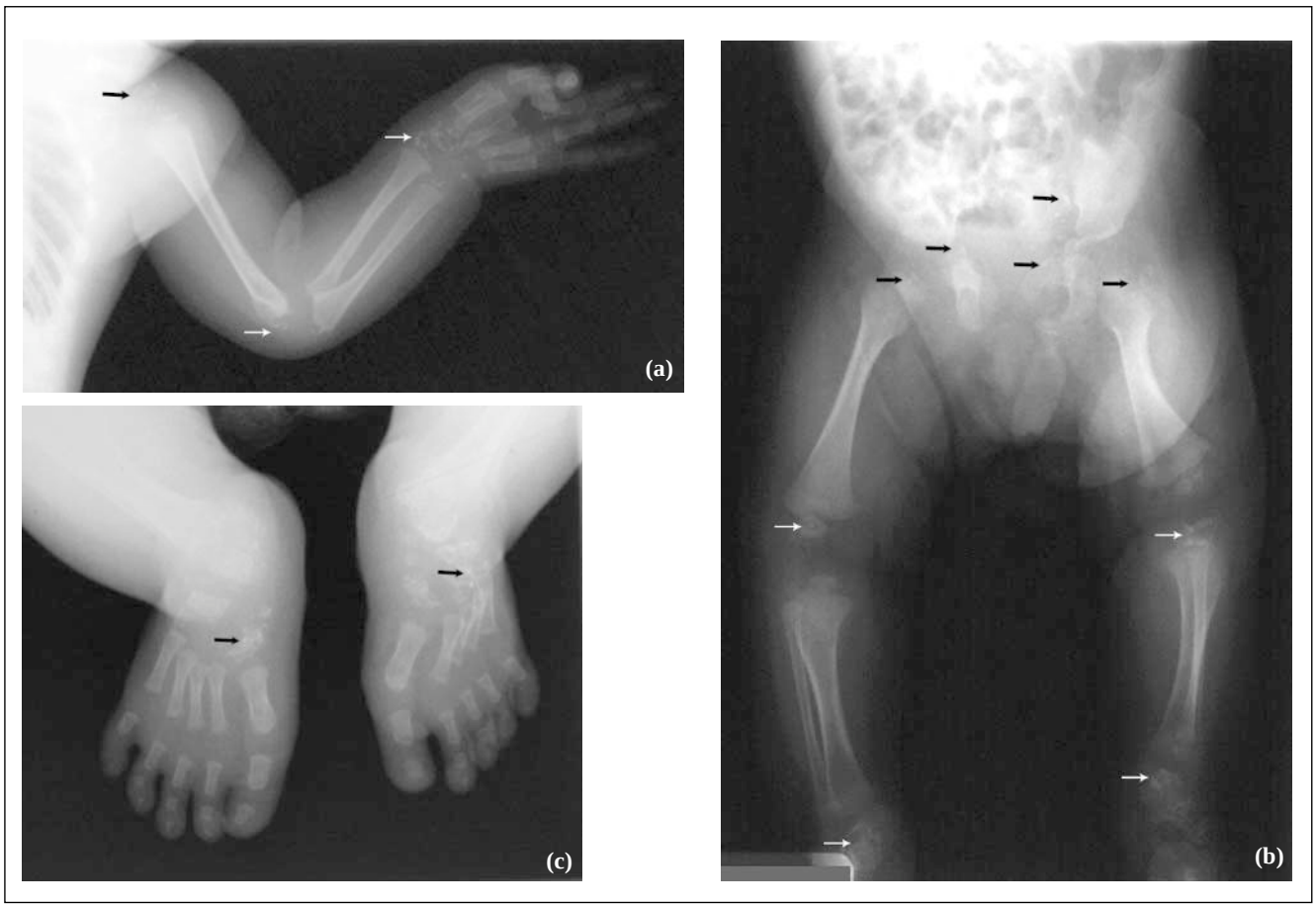

Figure 2 Punctate calcifications distributed at the ends of the long bones of the (a) upper limb, (b) pelvis and lower limbs, and (c) feet.

Table 1 Results of sterol metabolite analysis with plasma specimens obtained at 5 days and 4 months of age.

	5 days	4 months	Control	Ref. range (μg/ml)
8-dehydrocholesterol	26.8	7.2	undetectable	<0.01
Cholest-8(9)-en-3β-ol	89.2	15.9	undetectable	<0.01

nucleotide polymorphism (SNP) that does not lead to a change in amino acid coding.

Discussion

CDPX2 is a rare skeletal dysplasia of X-linked dominant inheritance. This is the first reported case in Chinese that has been confirmed by both biochemical and molecular testing. The major differential diagnoses were the autosomal recessive forms of rhizomelic chondrodysplasia punctata (RCDP1, RCDP2 and RCDP3). The differentiating clinical features were the asymmetrical limb shortening, the curvilinear pattern of alopecia and ichthyosis, and the lack of coronal clefting of vertebrae in CDPX2. Biochemical investigations for RCDP1, the commonest among the autosomal recessive forms, require red blood cell plasmalogen and plasma phytanic acid assays. For CDPX2, sterol metabolite analysis shows, as in the present case, elevation of cholest-8(9)-en-3β-ol, the immediate precursor of the defective enzyme, and 8-dehydrocholesterol, which in turn is a metabolite of cholest-8(9)-en-3β-ol. However, it is not yet understood why a defect in cholesterol biosynthesis leads to abnormal ossifications in the cartilage

matrix that is the cardinal feature shared by different forms of chondrodysplasia punctata. In fact, this feature is also present in several other cholesterol biosynthesis disorders like HEM dysplasia, CHILD syndrome, and Smith-Lemli-Opitz syndrome.

There were 55 *EBP* gene mutations reported to date.[4-14] They were heterogeneous and scattered throughout the gene. Furthermore all were small mutations including nonsense, missense, deletions or insertions, and splice site mutations. Only one case of in-frame deletion was previously reported, with deletion of nucleotides 293-295 in exon 2, leading to deletion of the 62nd amino acid, arginine, in the protein product.[7] Our patient represents the second case with such in-frame deletion. However, it is hard to determine how deletion of a single amino acid in the first transmembrane domain translates to the biochemical defect and the clinical phenotype. As this is a de novo mutation, the risk of recurrence to subsequent siblings is low. However, rare cases of gonadal mosaicism in one of the parents have been ascertained previously.[8] Therefore, the option of prenatal diagnosis in future pregnancies by molecular testing should be discussed with the parents.

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