OBSERVATION

Evidence of Postzygotic Mosaicism in a Transmitted Form of Conradi-Hünermann-Happle Syndrome Associated With a Novel *EBP* Mutation

Fanny Morice-Picard, MD; Elise Kostrzewa, MD; Claude Wolf, MD; Pascale Benlian, MD, PhD; Alain Taïeb, MD, PhD; Didier Lacombe, MD, PhD

Background: X-linked dominant chondrodysplasia punctata, also known as Conradi-Hünermann-Happle syndrome, is a rare skeletal dysplasia characterized by short stature, craniofacial defects, cataracts, ichthyosis, coarse hair, and alopecia. Conradi-Hünermann-Happle syndrome is caused by mutations in the gene *EBP* encoding Δ^8-Δ^7 sterol isomerase emopamil-binding protein. Random X-inactivation could account for the intrafamilial variability of the phenotype of X-linked dominant chondrodysplasia punctata.

Observations: We describe a girl with clinical features of X-linked dominant chondrodysplasia punctata. Biochemical analysis showed an abnormal sterol profile consistent with a defect in Δ^8-Δ^7 sterol isomerase. Molecular studies confirmed the diagnosis by identifying a novel heterozygous missense *EBP* mutation (c.199C>T; p.Cys67Arg). The mutation was not detectable on genomic DNA extracted from blood lymphocytes in both parents. The mother presented with an erythematous and ichthyosiform skin lesion. *EBP* analysis of DNA extracted from a lesional skin biopsy revealed the presence of p.Cys67Arg mutation.

Conclusion: To our knowledge, we report the first molecular confirmation of postzygotic mosaicism on an ichthyosiform skin lesion in the mother of a girl with X-linked dominant chondrodysplasia punctata associated with a novel *EBP* mutation.

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Author Affiliations: Service de Génétique Médicale (Drs Morice-Picard and Lacombe) and Service de Dermatologie et Dermatologie Pédiatrique, Centre de Référence pour les Maladies Rares de la Peau (Drs Morice-Picard, Kostrzewa, and Taïeb), Université de Bordeaux 2, Centre Hôpitalier Universitaire (CHU) de Bordeaux, Bordeaux, France; Faculté de Médecine Pierre et Marie Curie (UPMC), Université Paris 6, Paris, France (Dr Wolf); and Unité de Médecine Moléculaire du Métabolisme Lipidique, Faculté de Médecine, Université Lille 2, Lille, France (Dr Benlian).

X-LINKED DOMINANT CHONdrodysplasia punctata (CDPX2), also known as Conradi-Hünermann-Happle syndrome (OMIM 302960), is a disorder characterized by punctate chondrodysplasia, asymmetric shortness of limbs, linear ichthyosis, and cataracts. At birth, skin might be erythrodermic with a collodion baby phenotype and hyperkeratosis along the Blaschko lines. These features lead to atrophic hypopigmentary lesions and patchy areas of alopecia. Osseous anomalies are almost always present, often occurring with asymmetric shortness of limbs, vertebral defects with scoliosis, and epiphyseal stippling on radiographs. CDPX2 is caused by mutations in the *EBP* (emopamil-binding protein) gene (RefSeq, NM_006579).[1] This gene is located on chromosome Xp11.2-p11.23 and encodes a 3β-hydroxysterol-Δ^8-Δ^7-isomerase, which plays a major role in cholesterol biosynthesis pathway. Mutations in this gene lead to increased levels of cholesterol precursors cholesta-8-en-3β-ol and 8(9)-dehydrocholesterol. CDPX2 arises almost exclusively in girls, since it seems to be lethal early in boys. Phenotypic variability and asymmetrical anomalies have been related to random X-inactivation and to postzygotic mosaicism.[2] To our knowledge, we report the first molecular confirmation of postzygotic cutaneous mosaicism in an otherwise asymptomatic mother of a girl affected with CDPX2.

For editorial comment see page 1094

METHODS

PROFILING OF SERUM STEROLS

Gas chromatography–mass spectrometry analysis of circulating sterols was carried out as previously indicated.[3]

DNA ANALYSIS

Following informed consent for genetic testing, genomic DNA was extracted from peripheral blood leukocytes by a phase exchange

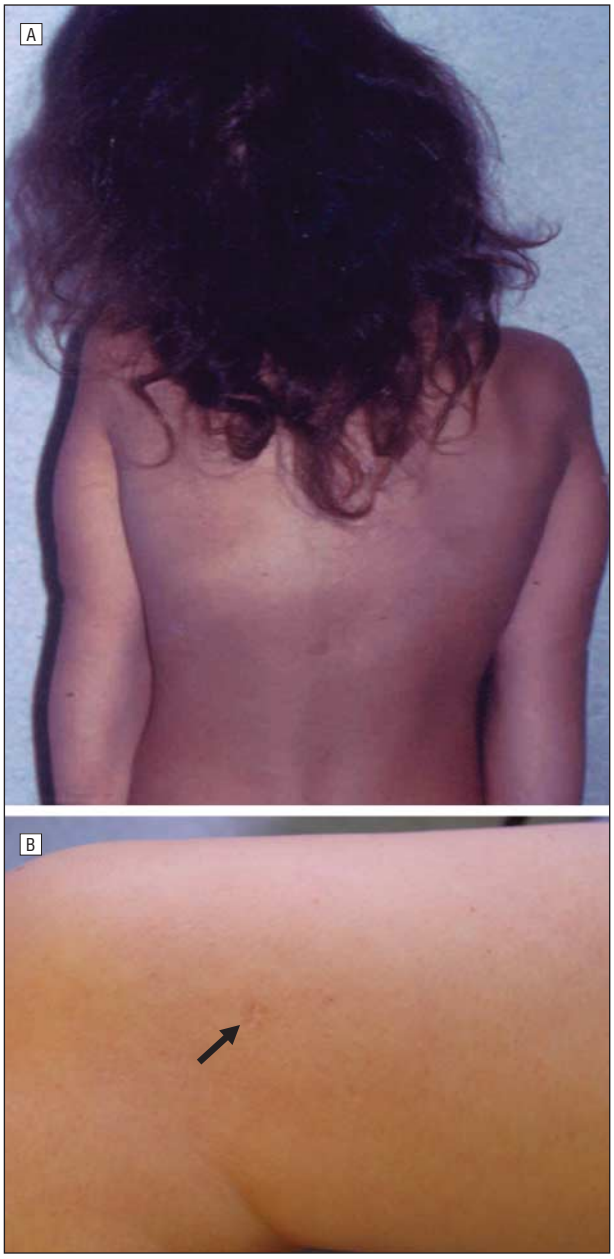

Figure 1. A, Short humerus, scoliosis, and patchy alopecia at age 10 years; B, erythematous and ichthyosiform lesion on the mother's right knee (arrow).

method (Puregene; Qiagen Inc, Valencia, California). DNA was extracted from mother's skin using an ion exchange column method, applying appropriate reagents for hard tissue DNA extraction (QIAamp DNA Mini Kit, Qiagen Inc). The coding sequence and flanking intronic sequences of the *EBP* gene were amplified by polymerase chain reaction (PCR) using the following primers: Ex2F CTT-CCT-GCC-TAT-ACA-CAC-GC, Ex2-R AGC-AAA-TCC-CAT-CCC-ACA-GC; Ex3-4F GTG-TGT-GTT-CCT-TTC-ACT-GC, Ex3-4R CAT-CTG-TGT-CTG-TGG-ATC-CC; Ex5F AAG-GTG-TGA-GCT-CTC-CTG-AG, Ex5R GAC-TAG-ACT-CTT-CTG-GCA-GG. Exonic and periexonic regions were sequenced from PCR products, on forward and reverse strands, by the Big-Dye terminator cycle-sequencing protocol on a 16-capillary DNA sequencer (ABI 3130; Applied Biosystems, Villebon Sur Yvette, France) as previously described.[4] The GenSearch software (http://www.gensource.com) was used to analyze raw sequence data for mutation detection and estimation of the deleterious potential of identified mutations against HUGO criteria and public genetic databases (http://www.phenosystems.com). In addition, several prediction algorithms (POLYPHEN [http://genetics.bwh.harvard.edu/pph], PMut [http://mmb2.pcb.ub.es:8080/PMut/], SIFT [http://blocks.fhcrc.org/sift], and SNPs3D [http://www.snps3d.org]) were used for computed estimation of the potential of a point mutation to induce functional changes on the protein, as previously described.[5] Any newly identified amino acid substitution was considered as deleterious if at least 3 algorithms provided consistent predictions.

DNA was extracted from blood leukocytes of 150 French healthy volunteer blood donors randomly selected over a period overlapping that of patient and family investigation. Genomic DNA was extracted and the *EBP* gene was analyzed according to the same procedures as described in the preceding paragraph. Results were used as control sequences from the referral population.

RESULTS

CLINICAL FINDINGS

We describe a 10-year-old girl born at term after an uneventful pregnancy. She was the first child of unrelated parents. Family history was uninformative. The mother had had 2 spontaneous abortions. The child was first assessed at birth owing to ichthyosis along the lines of Blaschko on the limbs and back associated with patchy alopecia. Radiographs showed epiphyseal punctate calcifications and a mild scoliosis. These findings led to the diagnosis of CDPX2 syndrome. Subsequent evaluation at 10 years showed growth delay (−2 SD), scoliosis, patchy alopecia, linear atrophic scars, and a moderate conductive bilateral deafness (**Figure 1**A). Ophthalmologic examination revealed lens opacities.

Parents were asymptomatic; however, careful examination of the skin of the mother showed an erythematous ichthyotic lesion on the right knee (Figure 1B). Findings from her ophthalmologic examination were normal. Two skin biopsies, one in lesional skin and the other in healthy skin, were performed.

PROFILING OF SERUM STEROLS

Gas chromatography–mass spectrometry analysis of circulating sterols was carried out in the proband and revealed elevated levels of 8-dehydrocholesterol and cholesta-8(9)-en-3β-ol (0.009 g/L and 0.039 g/L, respectively).

MUTATION ANALYSIS

Genomic DNA from peripheral blood leukocytes from the propositus and both parents was analyzed at the *EBP* locus. In the proband, a novel heterozygous missense mutation was identified in exon 2 (c.119T>C; p.Cys67Arg). The mutation was not found in DNA extracted from peripheral blood from both parents (**Figure 2**). Molecular analyses of genomic DNA extracted from skin biopsy specimens revealed the presence of a minor signal indicative of the presence of the mutation as found in the daughter with CDPX2 syndrome and in lesional skin from the mother, whereas there was no detectable mutation

in healthy skin. This confirmed the presence of postzygotic cutaneous and, almost certainly, gonadal mosaicism of the disorder in the mother.

COMMENT

CDPX2 is caused by mutations in the *EBP* gene located at Xp11.22-p11.23 and encoding for a Δ^8-Δ^7 sterol isomerase emopamil-binding protein,[6] a central component of cholesterol biosynthesis that catalyzes the conversion of 8(9)-cholestanol into lathosterol (5-a-cholest-7-en-3-b-ol to 5-a-cholest-8-en-3-b-ol). More than 60 deleterious mutations[7-9] have been described in this *EBP* gene. Functional defect of the mutated protein leads to accumulation of 8-dehydrocholesterol and 8(9)-cholestanol in plasma and tissues of the patients. Indeed, the proband described herein had elevated plasma levels of both these cholesterol precursors. The p.Cys67Arg mutation found in her family was not, to our knowledge, previously described in any disorder of cholesterol biogenesis. However, it disrupts a highly conserved residue located within the second membrane-spanning domain of the enzyme. This domain plays a structural role on catalytic site integrity of Δ^8-Δ^7 sterol isomerase.[1] In addition, 3 different algorithms used for computed estimation of the potential of this mutation to disturb protein function gave consistent conclusions regarding a probable deleterious effect of an arginine replacing the natural cysteine residue. Finally, this mutation was absent from 300 chromosomes from healthy volunteer blood donors, suggesting that this mutation was causative of the CDPX2 phenotype in the child and of the ichthyotic lesions of the skin in her mother.

The disorder mainly occurs in girls, since it is lethal in hemizygous boys. A few affected boys have been reported. Their survival was attributable to the presence of a somatic mosaicism or of a 47, XXY karyotype.[10,11] The clinical presentation is highly variable. The diverse phenotypes within the family and the asymmetry of the disease manifestations (limbs and cataracts) are explained by differences in expression of the mutated allele caused by random X-chromosome inactivation.[2,6,12] Thus, no correlation between the nature of the mutation and phenotype is ascertained.

CDPX2 mutations have occurred de novo and may be transmitted to the next generation. Phenotype variability can also be explained by postzygotic mosaicism. Such mutations were identified in boys with a moderate form of CDPX2 syndrome.[10] Moreover, postzygotic mosaicism was assumed in families with 2 affected children and healthy parents. In the mother of the family we examined herein, the presence of a small erythematous ichthyosiform skin lesion let us hypothesize postzygotic cutaneous mosaicism. The disease-causing mutation was not found in lymphocytes of the mother but was present in lesional skin, indicating a transmitted form of the disorder and indicative of gonadal mosaicism.

To our knowledge, this is the first description of gonadal mosaicism with an identified mutation on a skin biopsy specimen of the transmitter mother. Germline and somatic mosaicism have already been described in a healthy father of an affected child, with a mutation detected in sperm and hair follicles but absent in lymphocytes.[13] It was also hypothesized to be present in the grandmother of a girl with CDPX2 syndrome presenting only with cataract but without molecular confirmation.[14] These data must be taken into account for genetic counseling. We suggest that, besides ophthalmological evaluation for sectorial cataracts, a thorough skin examination is performed in all mothers of children born with CDPX2. Recurrence risk for future pregnancies depends on the rate of mosaicism in germ cells, which is impossible to specify.

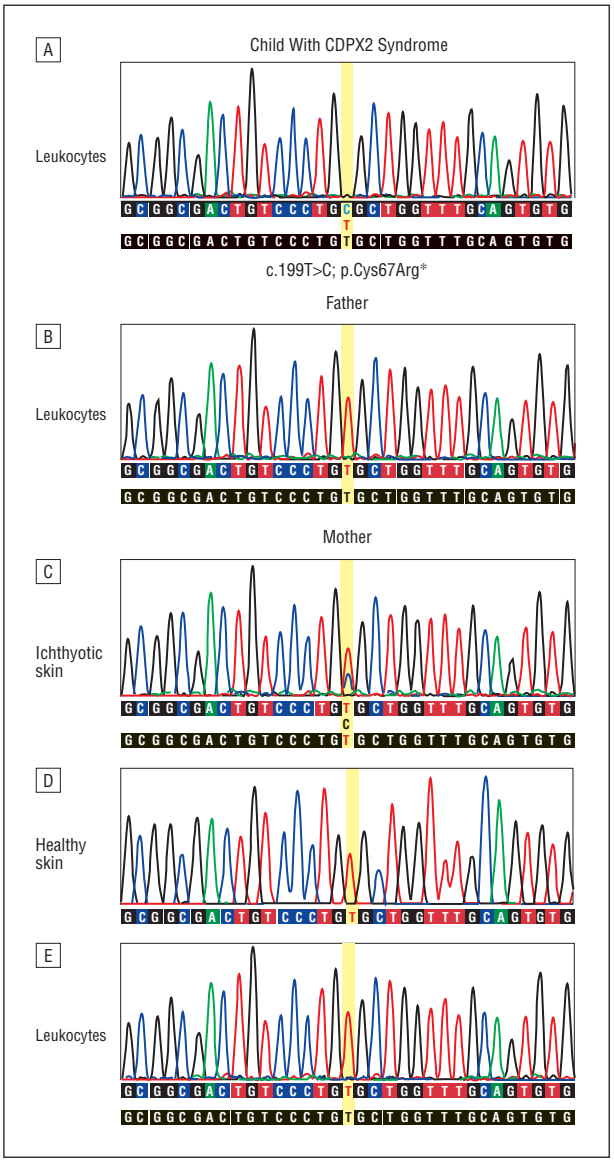

Figure 2. DNA analysis of the *EBP* gene in the proband and her parents. The mutation position is highlighted in yellow on DNA sequence tracks. The reference genomic sequence is represented in black at the bottom; above are DNA sequences identified in test DNA samples. Analysis of blood leukocytes from proband (A) and healthy father (B). Analysis of lesional skin (C), healthy skin (D), and blood leukocytes (E) in otherwise healthy mother. *Mutation found in Exon 2.

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Correspondence: Fanny Morice-Picard, MD, Service de Génétique Médicale, Hôpital Pellegrin ESF 3° Etage, 33076

Bordeaux CEDEX, France (fanny.morice-picard@chu-bordeaux.fr).

Author Contributions: Dr Morice-Picard had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Morice-Picard and Taïeb. *Acquisition of data:* Morice-Picard, Kostrzewa, Wolf, Benlian, Taïeb, and Lacombe. *Analysis and interpretation of data:* Morice-Picard, Kostrzewa, and Benlian. *Drafting of the manuscript:* Morice-Picard and Taïeb. *Critical revision of the manuscript for important intellectual content:* Morice-Picard, Kostrzewa, Wolf, Benlian, Taïeb, and Lacombe. *Administrative, technical, and material support:* Wolf. *Study supervision:* Taïeb and Lacombe.

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Correction

Errors in Byline, Author Affiliations, and Author Contributions. In the Study titled "Dermoscopic Features of Skin Lesions in Patients With Mastocytosis" by Vano-Galvan et al, published in the August issue of the *Archives* (2011;147[8]:932-940), errors occurred in the byline and the Author Affiliations section on page 932 and in the Correspondence and Author Contributions sections on page 939. In the byline, the names of 2 of the coauthors should have read "Elena De las Heras, MD, PhD" and "Maria N. Plana, MD, PhD." The corrected spellings of those surnames also should have been reflected in the Author Affiliations and Author Contributions sections.