Complete Maternal Isodisomy of Chromosome 3 in a Child with Recessive Dystrophic Epidermolysis Bullosa but No Other Phenotypic Abnormalities

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The mechanobullous disease Hallopeau–Siemens recessive dystrophic epidermolysis bullosa (HS-RDEB) results from mutations in the type VII collagen gene (COL7A1) on chromosome 3p21.31. Typically, there are frameshift, splice site, or nonsense mutations on both alleles. In this report, we describe a patient with HS-RDEB, who was homozygous for a new frameshift mutation, 345insG, in exon 3 of COL7A1. However, sequencing of parental DNA showed that although the patient’s mother was a heterozygous carrier of this mutation, the father’s DNA contained only wild-type sequence. Microsatellite marker analysis confirmed paternity and genotyping of 28 microsatellites spanning chromosome 3 revealed that the affected child was homozygous for every marker tested with all alleles originating from a single maternal chromosome 3. Thus, the HS-RDEB phenotype in this patient is due to complete maternal isodisomy of chromosome 3 and reduction to homozygosity of the mutant COL7A1 gene locus. To our knowledge, there are no published reports of uniparental disomy (UPD) in HS-RDEB; moreover, this case represents only the third example of UPD of chromosome 3 to be reported. The severity of the HS-RDEB in this case was similar to other affected individuals and no additional phenotypic abnormalities were observed, suggesting an absence of maternally imprinted genes on chromosome 3.


INTRODUCTION
Recessive dystrophic epidermolysis bullosa (RDEB) is a mechanobullous disorder associated with defective anchoring fibrils at the dermal–epidermal junction and abnormalities in type VII collagen gene and protein expression (Christiano et al., 1997a; Fine et al., 2000). The molecular pathology of RDEB involves loss-of-function mutations in the COL7A1 gene on chromosome 3p21.31 (Christiano et al., 1994). Typically, the severe Hallopeau–Siemens (HS) subtype of RDEB (OMIM 226600) results from nonsense, frameshift, splice site or, less commonly, missense mutations on both COL7A1 alleles (Jarvikallio et al., 1997; Whittock et al., 1999; Uitto and Richard, 2004). Clinically, HS-RDEB is associated with widespread mucocutaneous blisters and erosions, mutilating scarring and premature mortality, commonly from squamous cell carcinomas (Fine et al., 2000; Mallipeddi, 2002).

Genetic counseling based on Mendelian principles usually advises that parents of affected offspring are asymptomatic heterozygous carriers with a 25% risk of recurrence in subsequent pregnancies, although a number of de novo COL7A1 mutations have been reported (Uitto and Richard, 2004). Delineation of de novo mutations that are not present in parental germline may substantially reduce, but not completely abolish, the risk of recurrence. A further possibility, although not yet reported in RDEB, that has implications for the accuracy of genetic counseling and risk of recurrence is when molecular analysis discloses uniparental disomy (UPD), a term used to denote the inheritance of both copies of a chromosome pair from just one parent (paternal or maternal) (Engel, 1980). Uniparental heterodisomy refers to the presence of a pair of chromosome homologs, whereas uniparental isodisomy describes two identical copies of a single homolog, and meroisodisomy is a mixture of the two (Kotzot, 2001; Siegel and Slavotinek, 2005). In essence, uniparental isodisomy allows two copies of a recessive mutation to be transmitted from a heterozygous carrier parent.

We report the first case of HS-RDEB due to UPD, specifically complete maternal isodisomy, with reduction to homozygosity of the mutant COL7A1 gene. Moreover, this is
only the third reported example of UPD of chromosome 3 (Hoffman et al., 2004; Schollen et al., 2005).

RESULTS
The clinicopathological features of the blistering neonate support a diagnosis of HS-RDEB
The proband was the first-born son of healthy unrelated parents from Hong Kong. The mother was 34 years old and the father was aged 35. There was no family history of blistering skin diseases. The pregnancy, labor, and delivery were uncomplicated and the proband was born at 39 weeks gestation weighing 2,570 g (5th centile). At birth, there were extensive erosions at acral sites and on his legs (Figure 1a and b). He was otherwise clinically normal with no dysmorphic features and cytogenetic analysis showed a normal 46XY karyotype. Immunofluorescence microscopy demonstrated type IV collagen immunostaining to the roof of a skin blister (Figure 1c) and a complete absence of immunoreactivity for type VII collagen (Figure 1d), but normal labeling intensities for all other basement membrane components. These investigations are consistent with a diagnosis of HS-RDEB.

Now aged 1 year, he continues to have fragile skin with widespread trauma-induced blistering and erosions, more prominent on his hands and face; however, with supportive medical care he is thriving and is on the 50th centile for both weight and height. Complete assessment of his psychomotor development shows that he has achieved his appropriate developmental milestones and no physical abnormalities, other than the HS-RDEB, have been noted.

The molecular basis of the HS-RDEB is a new homozygous frameshift mutation, 345insG, in exon 3 of COL7A1
Direct automated sequencing revealed that the affected child was homozygous for a single-nucleotide insertion mutation, 345insG, in exon 3 of COL7A1 (Figure 2a), resulting in a premature termination codon 36-bp downstream. This mutation provides an explanation for the complete absence of type VII collagen immunolabeling on the skin biopsy (Figure 1d) and, although specific to this family, this mutation is fully consistent with HS-RDEB. Examination of the maternal DNA revealed that the mother was a heterozygous carrier (Figure 2b). The father’s DNA, however, showed only wild-type sequence (Figure 2c). Thus, the affected child was homozygous for the frameshift mutation, 345insG, and at least one allele was inherited from the mother.

Microsatellite analysis identifies complete maternal isodisomy of chromosome 3 and excludes non-paternity
To establish the origin of the affected child’s second mutant allele, haplotype analysis of the COL7A1 locus was initially performed based on inheritance of seven highly heterozygous microsatellite markers within 2 cM of the gene. These microsatellites comprised: D3S3678, D3S1581, D3S3629, D3S1568, D3S3026, D3S1578, and D3S1289 (see Table 1). The results showed that the affected child was homozygous for all seven markers, three of which (D3S3678, D3S1581, and D3S1578) showed an absence of a paternally derived
chromosome 3, and one marker (D3S3678) was fully informative for the child inheriting two copies of the mutated maternal allele. To examine the inheritance of the mutated alleles in the affected child further and to explore the possibility of UPD in this family, extensive genotype analysis of the entire chromosome 3 was performed using 21 additional microsatellite markers along the entire chromosome 3 (Table 1). The affected child was homozygous for all 21 of these markers, 10 of which were fully informative for inheritance of two copies of a single maternal chromosome 3 in the affected child. For the non-chromosome 3 markers, there were no discrepancies in the segregation of maternal and paternal alleles to the affected child, thus excluding non-paternity (data not shown). Normal karyotyping ruled out monosomy of chromosome 3. The results in this family are consistent with the inheritance of two identical copies of a single chromosome 3 homolog from the mother, thus indicating complete maternal isodisomy as the molecular basis of the HS-RDEB in this family.

**DISCUSSION**

UPD (isodisomy or meroisodisomy) with homozygosity of recessive alleles is being increasingly recognized as the molecular basis for several autosomal recessive disorders (Zlotogora, 2004). In fact, there have been over 35 reported cases of recessive diseases resulting from UPD (Kotzot and Utermann, 2005), although only two have involved chromosome 3. The first was a case of Fanconi Bickel syndrome

<table>
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<th>Locus</th>
<th>Cytogenetic location</th>
<th>Mother</th>
<th>Affected child</th>
<th>Father</th>
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</table>

The data show that the affected child has inherited two copies of a single maternal chromosome 3 (highlighted in bold). Marker order and cytogenetic location are derived from USCS Genome Bioinformatics (http://genome.ucsc.edu/); fully informative markers are indicated by asterisks (*).
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(OMIM 227810) caused by maternal isodisomy of chromosome 3 and reduction to homozygosity of a mutation in the glucose transporter gene GLUT2 (Hoffman et al., 2004). The second was a patient with a congenital disorder of glycosylation type Id (OMIM 601110) caused by a homozygous mutation in a mannosyl transferase gene, ALG3, resulting from a de novo mutation in combination with segmental maternal isodisomy (Schollen et al., 2005). Our case of HS-RDEB represents the third case of UPD of chromosome 3 and the second case of complete maternal isodisomy for this chromosome. However, this is not the first case of epidermolysis bullosa to result from UPD. Indeed, there have been four reported cases, all involving chromosome 1 in patients with Herlitz junctional epidermolysis bullosa (OMIM 226700). Three of these cases resulted from reduction to homozygosity of a mutation in the LAMB3 gene (locus 1q32.2) (Pulkkinen et al., 1997; Takizawa et al., 1998; Fassihi et al., 2005) and one involved a mutation in LAMC2 (1q25.3) (Takizawa et al., 2000).

For certain chromosomes, UPD can also result in distinct phenotypes depending on the parental origin of the chromosomes, a phenomenon known as genomic imprinting. This parent-of-origin specific gene expression is determined by epigenetic modification of a specific gene or, more often, a group of genes, such that gene transcription is altered and only one inherited copy of the relevant imprinted gene(s) is expressed in the embryo. (Swales and Spears, 2005). This means that during development, the parental genomes function unequally in the offspring, the most common examples of genomic imprinting being Prader–Willi (OMIM 176270) and Angelman (OMIM 105830) syndromes, which can result from maternal or paternal UPD for chromosome 15, respectively (Nicholls and Knepper, 2001). These phenotype abnormalities commonly associated with UPD for chromosomes with imprinting are intrauterine growth retardation, developmental delay, and reduced stature (Miozzo and Simoni, 2002; Coan et al., 2005). For the chromosome 3 UPD in our case of HS-RDEB, the child did have a low birth weight; however, it is plausible that any intrauterine growth restriction could simply have been due to the blistering skin disease (Fox et al., 2003). Moreover, physical examination over the first year of this child’s life has not shown any significant growth or developmental abnormalities, observations that suggest an absence of clinically significant maternally imprinted genes on chromosome 3, although clearly further clinical follow-up will be necessary to confirm this initial impression. Indeed, thus far there have been no reports of imprinted genes on human chromosome 3 and normal phenotype during the first year of life other than the HS-RDEB in this case of maternal isodisomy suggests that maternally derived genes on human chromosome 3 are not imprinted.

MATERIALS AND METHODS

Immunofluorescence microscopy

Following ethical approval, informed consent and in compliance with the Declaration of Helsinki Principles, a skin biopsy was taken under local anesthesia from clinically uninvolved skin on the buttock. Indirect immunofluorescence staining was performed on 5 µm cryosections of skin with the monoclonal antibodies LH7:2 (NC-1 domain of type VII collagen, a gift from Dr I.M. Leigh) (Leigh et al., 1988), GB3 (conformational epitope on γ2 chain of laminin 5, Oxford Biotechnology Ltd (Kidlington, UK) (Verrando et al., 1991)) and COL94 (type IV collagen, Sigma, Poole, UK). The antibodies were diluted 1:1,000, 1:300, and 1:500, respectively, in phosphate-buffered saline/bovine serum albumin. Labeling procedures were as described previously (Kennedy et al., 1985). Fluorescein isothiocyanate-labeled anti-mouse and anti-rabbit secondary antibodies (Dako, Glostrup, Denmark) were used diluted 1:200 in phosphate-buffered saline/bovine serum albumin.

Mutation analysis

DNA was extracted from peripheral blood samples taken from the proband and his parents using standard protocols. Mutation detection strategy consisted of heteroduplex scanning by conformation-sensitive gel electrophoresis of all 118 exons of COL7A1. Laboratory protocols and details of the specific primers have been
described elsewhere (Christiano et al., 1997b). The corresponding PCR products showing heteroduplexes were subjected to bi-directional automated nucleotide sequencing using an ABI Prism 310 Genetic Analyzer.

Genotype analysis
For haplotype analysis of the COL7A1 locus, seven highly heterozygous microsatellite markers within 2 cM of the gene were initially tested (D1S3678, D3S1581, D3S3629, D3S1568, D3S3026, D3S1578, and D1S1289). In addition, 21 further microsatellite markers spanning the entire chromosome 3, approximately 10 cM apart, were analysed from the proband and his parents. Non-paternity was investigated using six non-chromosome 3 markers (D1S425, D1S2703, D1S205, D1S491, D6S291, and D6S276). All microsatellite markers were from the ABI Prism Linkage Mapping Set Version 2.5 (Applied Biosystems, Warrington, UK), were amplified with fluorescently labeled oligonucleotides, and used under conditions recommended by the manufacturer. Electrophoretic analysis was performed on an ABI Prism 310 Genetic Analyzer with Performance Optimized Polymer 4 (POP4) using Genescan software (Applied Biosystems). The allele sizes were analysed using Genetyper software (Applied Biosystems).

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
Funding support for this work was kindly provided by the Dystrophic Epidermolysis Bullosa Research Association (DeBRA UK). We are also grateful to the patient’s family for helping us compile this report.

REFERENCES


