Complete Paternal Uniparental Isodisomy of Chromosome 1: A Novel Mechanism for Herlitz Junctional Epidermolysis Bullosa

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Uniparental disomy denotes a situation when an individual has inherited two copies of a specific chromosome from a single parent. Uniparental disomy has been demonstrated to be involved in the pathogenesis of recessively inherited diseases in rare cases. Here we report a patient of Japanese origin with Herlitz junctional epidermolysis bullosa (OMIM no. 226700), who died at the age of 8 mo from complications of the disease. The mutation analysis revealed that the proband was homozygous for a nonsense mutation C553X in the LAMC2 gene encoding the γ2 chain of laminin 5. The father was a heterozygous carrier of this mutation whereas the mother had two normal alleles of this gene. The patient showed homozygosity for 15 known intragenic polymorphisms in the LAMC2 gene. Furthermore, genotype analysis, performed from the parents and the proband, using 16 microsatellite markers spanning the entire chromosome 1, revealed that the patient was homozygous for all markers tested, and that these alleles originated from the father. Among the 16 markers, eight were fully informative for the absence of the maternal chromosome 1 in the proband, suggesting that the patient had complete paternal isodisomy of this chromosome. Thus, the Herlitz junctional epidermolysis bullosa phenotype in this patient is caused by homozygous LAMC2 mutation C553X that is of paternal origin and results from nondisjunction and uniparental disomy involving monosomy rescue. This is a novel mechanism resulting in Herlitz junctional epidermolysis bullosa and has implications for assessment of the risk in subsequent pregnancies. ***Key words: basement membrane zone/blistering skin diseases/ laminin 5 mutations. J Invest Dermatol 115:307–311, 2000***

The Herlitz variant of junctional epidermolysis bullosa (H-JEB) is a severe blistering disease characterized by tissue separation within the dermal-epidermal basement membrane zone at the level of the lamina lucida. This condition characteristically results in early demise of the affected individuals, usually within the first year of life, due to complications of the blistering condition and associated extracutaneous manifestations (Fine *et al*, 1999). It has been well established that the molecular basis of H-JEB revolves around premature termination codon (PTC) mutations in the genes encoding laminin 5 subunit polypeptides, which result in the absence of laminin 5 in the cutaneous basement membrane zone (Pulkkinen *et al*, 1999). Specifically, PTC mutations have been disclosed in LAMA3, LAMB3, and LAMC2 genes, encoding the α3, β3, and γ2 subunit polypeptides, respectively, of laminin 5. These genes have been mapped to human chromosomes 18q11.2, 1q32, and 1q25–31, respectively (Ryan *et al*, 1994; Vailly *et al*, 1994). It should be noted, however, that the phenotypic severity of JEB can be modified by the type and combination of mutations in the laminin 5 as well as other basement membrane zone genes (Floeth and Bruckner-Tuderman, 1999; McGrath *et al*, 1999).

In general, the inheritance of H-JEB follows the Mendelian rule in an autosomal recessive pattern, and consequently, the parents of the affected individuals are obligate heterozygous carriers of the respective mutations. Recently, however, two cases with H-JEB were shown to result from maternal uniparental disomy (UPD) for chromosome 1 involving the isodisomic region spanning the mutant LAMB3 gene (Pulkkinen *et al*, 1997a; Takizawa *et al*, 1998a). UPD is defined as a situation whereby an individual has inherited two copies of a specific chromosome from a single parent. Specifically, the inheritance of a pair of chromosome homologs from a parent is defined as uniparental heterodisomy, whereas the inheritance of two identical copies of a single chromosome homolog from a parent is defined as uniparental isodisomy (Engel, 1993). In rare cases, recessive diseases have been shown to be associated with UPD (Ledbetter and Engel, 1995). In the two previously reported H-JEB cases caused by maternal UPD, the probands had inherited two identical copies of a segment of chromosome 1 spanning the LAMB3 locus. Therefore, in these

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Abbreviations: H-JEB, Herlitz junctional epidermolysis bullosa; PTC, premature termination codon; UPD, uniparental disomy.

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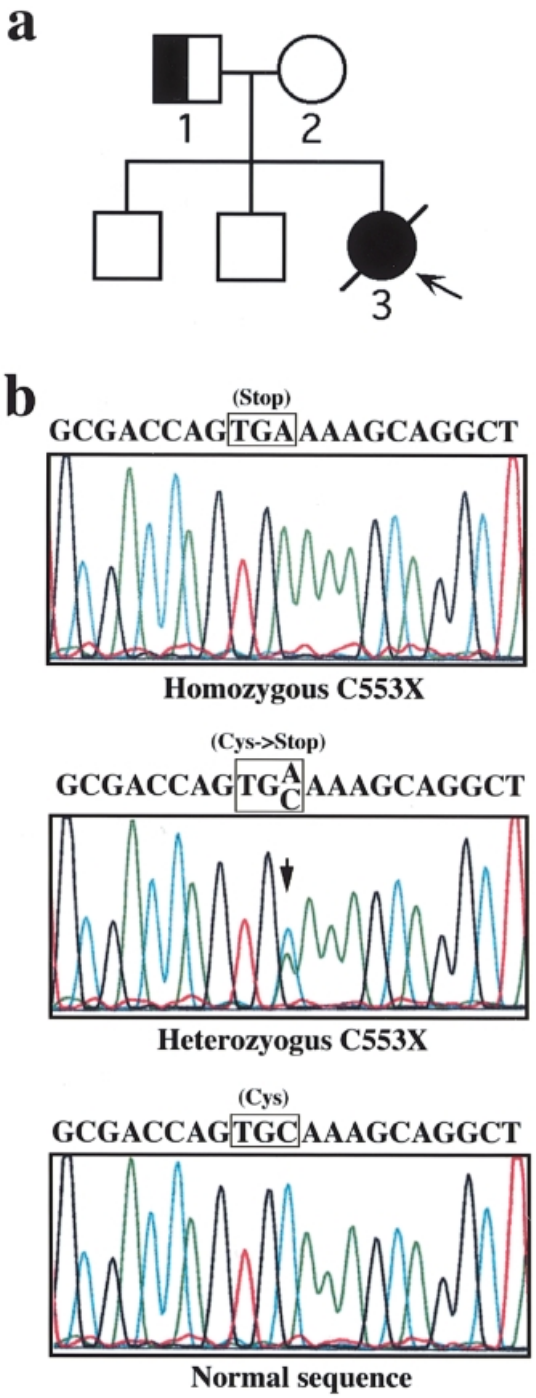

Figure 1. Mutation detection in the family with H-JEB. The proband, a 2-mo-old female, is indicated by an arrow in the pedigree. The parents and the two older brothers are clinically normal (*a*). Direct sequencing of exon 11 of the LAMC2 gene disclosed in the proband a homozygous C-to-A transversion at position 1776 (*b*, *upper panel*). This nucleotide substitution resulted in the change of a codon for cysteine to a PTC (TGC → TGA), a mutation designated as C553X. The father of the proband was a heterozygous carrier of this mutation (*b*, *center panel*), whereas the mother showed a normal sequence only (*b*, *lower panel*).

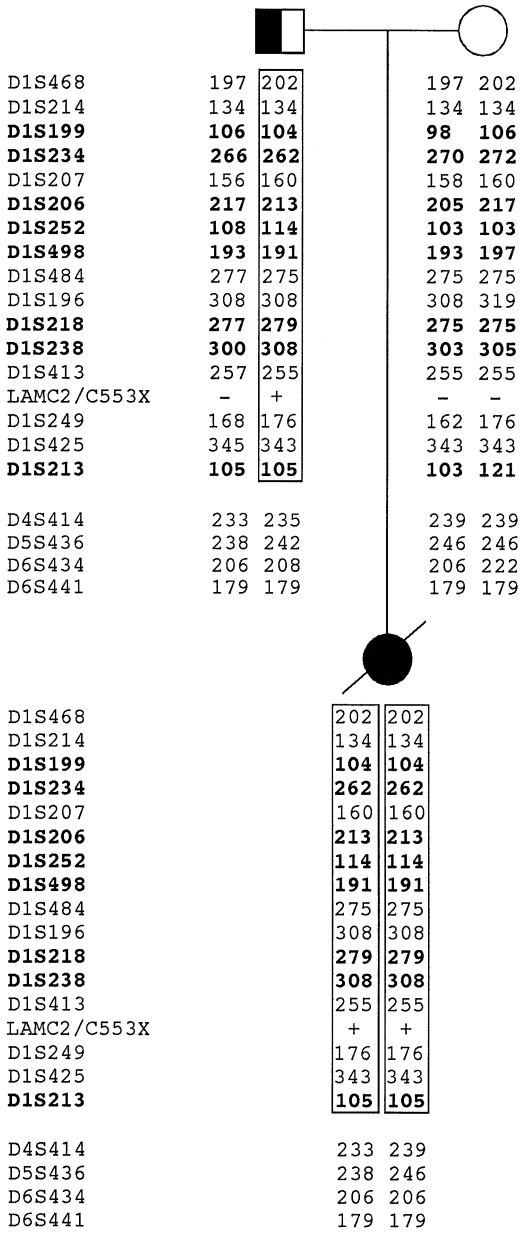

Figure 2. Genotype analysis of DNA from the proband and her parents using 16 polymorphic microsatellite markers in chromosome 1. Note that the proband was homozygous for eight markers along one copy of the paternal chromosome 1 homolog (boxed), and nine of the markers (in bold) were fully informative for the absence of the maternal chromosome 1 in the proband. Microsatellite markers corresponding to chromosomes 4, 5, and 6 indicated that the proband had inherited these chromosome homologs from both parents.

cases, the mutation inherited from the mother was reduced to homozygosity resulting in the H-JEB phenotype.

In this report we delineate a case of paternal UPD with mutations in the LAMC2 gene on chromosome 1q25–31, which we further fine-mapped by radiation hybrid panel. This is the first demonstration of paternal UPD in H-JEB, and the first UPD with mutations in LAMC2. In addition, this is the first description of complete isodisomy of chromosome 1.

MATERIALS AND METHODS

Clinical history and diagnostic features The proband, a 2-mo-old female, was the third child of unrelated, clinically normal parents of Japanese origin. There was no family history of blistering skin diseases, and specifically the older brothers of the proband were clinically normal (**Fig 1*a***). The proband had blistering of the skin and oral mucous membranes noted at birth. Immunofluorescence analysis of the skin of the proband revealed negative expression of laminin 5, as determined by the

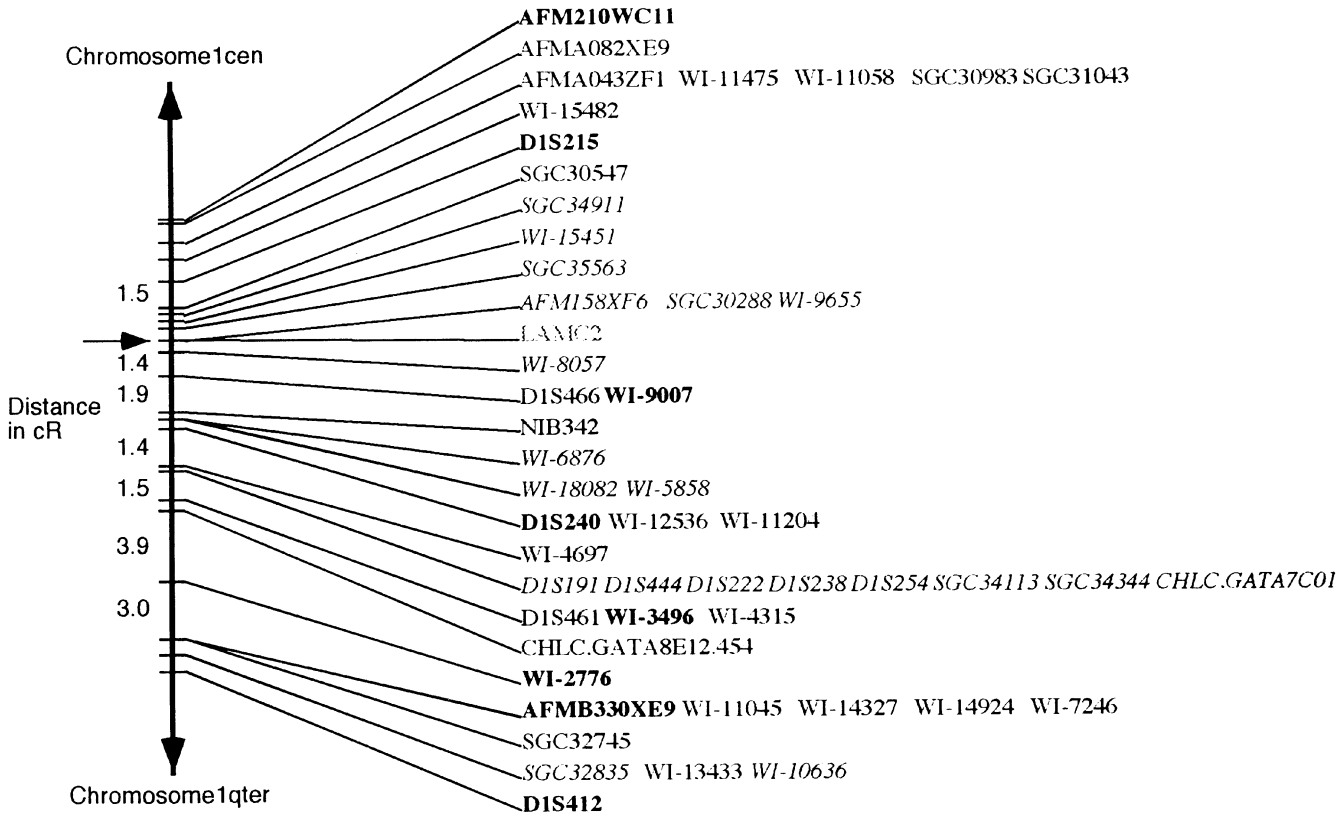

Figure 3. High resolution radiation hybrid map of the region flanking the LAMC2 locus on chromosome 1q. Distances are in centiRays (cR), and the framework markers are indicated in boldface. The LAMC2 locus (→) is flanked by polymorphic framework markers D1S215 and D1S240, which are about 5.2 cM apart.

monoclonal antibody GB3. Staining with the monoclonal antibody 19-DEJ-1 was also negative, although staining with the monoclonal antibody LH7.2, which recognizes type VII collagen, was normal. These findings are diagnostic for H-JEB. The proband died at the age of 8 mo from complications of the disease.

Mutation analysis DNA was extracted from peripheral blood samples of the proband and her parents using standard protocols. Mutation detection strategy consisted, first, of analysis of two recurrent LAMB3 mutations, W610X and Q166X, present in the Japanese population (Takizawa *et al*, 1998b). This was followed by heteroduplex scanning by conformation-sensitive gel electrophoresis of all exons of LAMB3 and exons 5, 8, 11, and 17 of LAMC2 (Pulkkinen *et al*, 1995, 1997b). The corresponding polymerase chain reaction (PCR) products showing heteroduplexes were subjected to automated nucleotide sequencing.

Genotype analysis For haplotype analysis of the LAMC2 locus, 15 intragenic two-allelic polymorphisms were tested from the parents and the proband (Kon *et al*, 1998). The polymorphic regions were amplified and analyzed by restriction enzyme digestion and agarose gel electrophoresis. In addition, 16 multiallelic microsatellite markers spanning the entire chromosome 1 were analyzed from the proband and her parents using fluorescence-labeled primers for amplification of the polymorphic regions (PE Biosystems, Foster City, CA). The PCR products were then analyzed on DNA sequencer ABI 377 using Genescan software. The allele sizes were analyzed using Genotyper software.

Radiation hybrid mapping of LAMC2 A 326 bp fragment spanning exon 14 and flanking intronic sequences of LAMC2 was amplified using the GeneBridge 4 radiation hybrid panel with the following primers: sense primer, 5′-TTGCTAACTCTATGCTGACC-3′; antisense primer, 5′-TCTACAAGTAGGTCTCCCAA-3′. The amplification conditions are described elsewhere (Pulkkinen *et al*, 1997b). The resulting data vector (00000 00001 00110 00000 00001 10000 00010 00010 00100 20000 00000 00000 00100 00010 01011 00000 01000 00001 001) was mapped by the use of LOD score > 17 by the RHMapper server, which was accessed on-line at the Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology (Whitehead Institute, 1999).

RESULTS AND DISCUSSION

Mutation identification The initial efforts to identify mutations in this family with H-JEB concentrated on LAMB3 due to the fact that the majority (≈80%) of all H-JEB mutations reside in this gene (Pulkkinen *et al*, 1999). Analysis of the recurrent Japanese LAMB3 mutations W610X and Q166X (Takizawa *et al*, 1998b), and heteroduplex scanning of all 23 exons of LAMB3, followed by direct sequencing of the corresponding PCR products, failed to identify pathogenetic mutations. Subsequently, analysis of LAMC2 was initiated by heteroduplex scanning of exons 5, 8, 11, and 17, which have been shown previously to harbor mutations in Japanese patients with H-JEB (Kon *et al*, 1998; Takizawa *et al*, 1998c). Direct automated sequencing of the corresponding PCR products revealed that the proband was homozygous for a nucleotide substitution, C-to-A in position 1776, corresponding to exon 11 of LAMC2. This transversion substitution resulted in change of the codon for cysteine to the termination codon for translation (TGC → TGA), and the mutation was designated as C553X. Examination of the parental DNA revealed that the father, as expected, was a heterozygous carrier of this C553X mutation (**Fig 1*b***). Surprisingly, however, the mother's DNA revealed the presence of two normal alleles only, identical to normal unrelated controls (**Fig 1*b***). Thus, the proband was homozygous for a PTC mutation (C553X), and at least one of the alleles was inherited from the father. This mutation has previously been reported in another Japanese H-JEB patient, but in that case the parents were clearly heterozygous carriers of this mutation, thus excluding UPD (Kon *et al*, 1998).

Elucidation of UPD To examine the inheritance of the mutated alleles in the proband, and to explore the possibility of UPD in this family, extensive genotype analyses for the LAMC2 locus and the entire chromosome 1 were performed. First, 15 biallelic single-base polymorphisms in the LAMC2 gene, located in chromosomal locus 1q25-q31 (Vailly *et al*, 1994), were examined by PCR amplification, followed by restriction enzyme digestions (Kon *et al*, 1998). The results indicated that the patient was homozygous for each marker tested, suggesting UPD as a potential mechanism for H-JEB. To examine this possibility in further detail, 16 microsatellite markers spanning the entire chromosome 1 were tested in the proband and her parents (**Fig 2**). The proband was homozygous for all 16 markers, and eight of the markers were fully informative for the absence of the maternal chromosome 1 in the proband. At the same time, microsatellite markers corresponding to chromosomes 4, 5, and 6 indicated inheritance of markers from both parents, thus confirming the maternal origin of DNA from individual 2 in **Fig 1**(***a***) (**Fig 2**). As monosomy of an entire autosomal chromosome has not been reported and may result in miscarriage in early pregnancy, it is likely that the proband had inherited two identical copies of a single chromosome 1 homolog from the father, thus representing complete paternal isodisomy. In addition, the proband did not display overt dysmorphic features at birth. Both parents were 36 y of age at the time of pregnancy. As the risk for nondisjunction increases with increasing parental age, this information provides further explanation for the occurrence of UPD in this family.

To assist in further linkage studies to ascertain the laminin 5 genes as candidates in JEB and to identify informative markers flanking the LAMC2 locus, we also fine-mapped LAMC2, which has been previously assigned to 1q25–31 by chromosomal *in situ* hybridization (Vailly *et al*, 1994). For this purpose, we used the GeneBridge 4 radiation human-hamster hybrid panel with primers amplifying exon 14 and flanking intronic sequences of LAMC2. By the use of the RHMapper program, the data vector was placed 4.60 cR from proximal framework marker D1S215 on chromosome 1, and was bound by a distal framework marker D1S240 (**Fig 3**). Both of these markers are CA-repeats with 8 and 10 different alleles with heterozygosities of 0.73 and 0.63, respectively (Genome Database homepage, 2000). It should be noted that previous radiation hybrid mapping placed the LAMC1 gene to the same interval (Williams *et al*, 1997). On the basis of these results and our previous radiation hybrid mapping data on LAMB3 (Pulkkinen *et al*, 1997a), the distance between LAMC2 and LAMB3 was estimated to be about 38 cM. It should be noted that the isodisomic regions in previously reported H-JEB cases caused by UPD did not involve the LAMC2 locus.

Implications of UPD in H-JEB Previously, we reported two H-JEB cases caused by maternal UPD with mutations in the LAMB3 gene (Pulkkinen *et al*, 1997a; Takizawa *et al*, 1998a). The case delineated in this study is the first paternal UPD resulting in H-JEB, and the first with mutations in LAMC2. Paternal UPD for chromosome 1 has been previously reported in a patient with pycnodysostosis, a rare autosomal recessive sclerosing skeletal dysplasia (Gelb *et al*, 1998). This patient was homozygous for markers near the centromer of chromosome 1, but was clearly heterozygous for markers towards both telomers, indicating partial isodisomy (meroisodisomy). In contrast, our case with H-JEB was homozygous for all microsatellite markers along chromosome 1, including telomeric and centromeric regions, suggesting complete isodisomy of chromosome 1. Thus, this case appears to be the first demonstration of a complete isodisomy of this chromosome.

The mechanism for the development of UPD in this case is likely to involve nondisjunction in meiotic division II in the mother, resulting in disomic and nullisomic gametes. Subsequent to fertilization of the nullisomic gamete with the paternal haploid gamete, the entire paternal chromosome 1 is duplicated, yielding two identical copies of this chromosome. This monosomy rescue results in homozygosity of the LAMC2 locus containing the paternal mutation C553X and in the H-JEB phenotype. This is clearly a novel mechanism for H-JEB, distinct from the two maternal UPD cases presenting with meroisodisomy of the region spanning the LAMB3 locus (Pulkkinen *et al*, 1997; Takizawa *et al*, 1998). In the previous cases, the nondisjunction potentially occurred during the first meiosis, and the UPD most probably resulted from trisomy rescue, as previously reported (Pulkkinen *et al*, 1997; Takizawa *et al*, 1998).

Elucidation of the mechanisms for H-JEB in this case has clear implications for genetic counseling for the risk of recurrence of H-JEB in this family. Specifically, the carrier risk for H-JEB mutations in the laminin 5 gene is 1 in 770 (Nakano *et al*, 2000), and therefore the risk of recurrence is expected to be extremely low, $\approx 4 \times 10^{-5}$, rather than 1 in 4, as would be the case in an autosomal recessive disease. At the same time, the frequency of UPD in chromosome 1 has been suggested to be approximately 1 in 200 (Field *et al*, 1998), again attesting to the low risk of recurrence of H-JEB through this mechanism.

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