

Maternal Uniparental Disomy of Chromosome 1 with Reduction to Homozygosity of the LAMB3 Locus in a Patient with Herlitz Junctional Epidermolysis Bullosa

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Summary

Junctional epidermolysis bullosa (JEB) is an autosomal recessive disorder characterized by blister formation at the level of the lamina lucida within the cutaneous basement-membrane zone. Classic lethal JEB (Herlitz type [H-JEB]; OMIM 226700) is frequently associated with premature-termination-codon mutations in both alleles of one of the three genes (LAMA3, LAMC2, or LAMB3) encoding the subunit polypeptides (α 3, β 3, and γ 2) of laminin 5. In this study, we describe a unique patient with H-JEB, who was homozygous for a nonsense mutation, Q243X, in the LAMB3 gene on chromosome 1 and who had normal karyotype 46,XY. The mother was found to be a carrier of the Q243X mutation, whereas the father had two normal LAMB3 alleles. Nonpaternity was excluded by use of 11 microsatellite markers from six different chromosomes. The use of 17 partly or fully informative microsatellite markers spanning the entire chromosome 1 revealed that the patient had both maternal uniparental merodisomy of a 35-cM region on 1q containing the maternal LAMB3 mutation and maternal uniparental heterodisomy of other regions of chromosome 1. Thus, the results suggested that reduction to homozygosity of the 1q region containing the maternal LAMB3 mutation caused the H-JEB phenotype. The patient was normally developed at term and did not show overt dysmorphisms or malformations. This is the first description of uniparental disomy of human chromosome 1.

Introduction

Uniparental disomy (UPD) is defined as a situation whereby an individual has inherited two copies of a specific chromosome from a single parent in a disomic cell line (Engel 1980; Niikawa and Kajii 1984). Specifically, the inheritance of a pair of chromosome homologues from one parent is uniparental heterodisomy, whereas the inheritance of two identical copies of a single chromosome homologue from one parent is defined as uniparental isodisomy. UPD in humans, caused primarily by meiotic nondisjunction events, is often associated with advanced maternal age, and is followed by trisomy or monosomy rescue (Engel 1993; Ledbetter and Engel 1995). Some UPDs have been described as a result of both maternal and paternal nondisjunction followed by complementation of a disomic gamete by a nullisomic gamete, which also reflects involvement of advanced paternal age, in this mechanism (Robinson et al. 1993; Abruzzo and Hassod 1995).

UPD for different chromosomes increasingly has been detected as a result of improved coverage of human chromosomes with highly polymorphic markers, and, so far, this chromosomal aberration has been described in humans for 16 autosomes, as well as for the X and Y chromosomes (Engel 1993; Ledbetter and Engel 1995; Benlian et al. 1996). Abnormal phenotypes associated with UPD can result from the presence of imprinted genes on the chromosome involved in this non-Mendelian inheritance mechanism or from reduction to homozygosity of the mutation associated with an autosomal recessive disorder. Furthermore, the effects of trisomy on the placenta or the fetus can cause various prenatal and postnatal abnormalities. Imprinting, which refers to differential gene expression depending on the parental origin of the chromosome, is thought to play a regulatory role in growth and development (Hall et al. 1990; Cassidy 1995). Uniparental inheritance of an imprinted gene(s) has been clearly demonstrated in UPD for chromosome 15 in patients with Angelman syndrome and Prader-Willi syndrome, and for chromosome 11 in pa-

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tients with Beckwith-Wiedemann syndrome and Wilms tumor (Ledbetter and Engel 1995).

The association of an autosomal recessive phenotype with UPD was first described in a patient with cystic fibrosis (Spence et al. 1988), followed by several other similar discoveries (Voss et al. 1989; Langlois et al. 1995). These cases demonstrated maternal UPD of chromosome 7, which was also discovered in a patient with osteogenesis imperfecta with a homozygous COL1A2 mutation (Spotila et al. 1992). A common additional feature in these patients was severe growth retardation, which was thought to reflect the presence of a maternally inherited imprinted gene(s) in chromosome 7 (Eggerding et al. 1994). In support of this hypothesis, a paternal UPD of chromosome 7 was demonstrated in a patient with autosomal recessive congenital chloride diarrhea, with no dysmorphic features, suggesting the absence of imprinted genes in paternally inherited chromosome 7 (Höglund et al. 1994). Furthermore, a maternal UPD for chromosome 14 was found in a patient with autosomal recessive rod monochromacy associated with several other abnormal features, including short stature, mild developmental delay, and some minimal dysmorphisms (Pentao et al. 1992). In this case, the disease gene for rod monochromacy subsequently was mapped to chromosome 14. The other clinical features, apart from rod monochromacy, were a result of a maternally inherited imprinted gene(s), as was further suggested by several additional studies (Ledbetter and Engel 1995). In addition to the conditions discussed above, involvement of UPD in the pathogenesis of other recessive disorders has been described elsewhere, including that of spinal muscular atrophy type III, for chromosome 5; complement component deficiencies, for chromosome 6; β -thalassemia, for chromosome 11; Bloom syndrome, for chromosome 15; and lipoprotein lipase deficiency, for chromosome 8 (Cassidy 1995; Benlian et al. 1996). In all these cases, the recessive phenotype resulted from the reduction to homozygosity of the mutant allele.

Junctional epidermolysis bullosa (JEB) is a clinically heterogeneous genodermatosis that is inherited in an autosomal recessive fashion and that shows ultrastructural abnormalities in the hemidesmosome-anchoring filament complex within the cutaneous basement membrane zone (Tidman and Eady 1986). The lethal Herlitz variant of JEB (H-JEB; OMIM 226700) is associated with premature-termination-codon (PTC) mutations in both alleles of one of the three genes (LAMA3, LAMB3, or LAMC2) encoding the constitutive polypeptide subunits of laminin 5, the major component within the dermal-epidermal basement membrane zone (Uitto et al. 1995; Uitto and Pulkkinen 1996). Two of these genes, LAMB3 and LAMC2, are mapped to the long arm of chromosome 1 (Vailly et al. 1994), whereas LAMA3 is

placed to the long arm of chromosome 18 (Ryan et al. 1994). In this study, we describe UPD of chromosome 1 in a patient with H-JEB, who was homozygous for a maternal nonsense mutation in the LAMB3 gene.

Patient, Material, and Methods

Patient

The proband was a newborn male with unrelated healthy parents who were of Scottish-Irish (mother) and German-Irish (father) descent and who had no family history of blistering skin diseases. The proband had two older, clinically unaffected sisters. Amniocentesis had demonstrated a normal karyotype of 46,XY in the proband. At birth, he was noted to have abnormal nails, and, within a few days after birth, he developed blisters first in the mouth and then covering the entire body surface. The proband died at the age of 2 mo, from complications of the disease. Epitope mapping of the blistered skin, by immunohistochemistry used as a diagnostic tool, demonstrated that the bullous pemphigoid antigen was located on the roof of the blister, whereas type IV collagen was located on the base of the blister cavity. These findings are consistent with blister formation at the level of the lamina lucida within the cutaneous basement membrane zone. On the basis of these findings, together with clinical examination, the proband was diagnosed as H-JEB.

Mutation Analysis

DNA from the proband and from his parents, isolated from peripheral blood, was subjected to mutation analysis of the LAMB3 gene, which was performed by PCR amplification of each exon of this gene (Pulkkinen et al. 1995), followed by heteroduplex analysis using conformation-sensitive gel electrophoresis (CSGE) (Ganguly et al. 1993). The PCR amplification products showing heteroduplexes were subjected to direct automated nucleotide sequencing (ABI).

For amplification of exon 8 of LAMB3, the following oligonucleotide primers were used: E8-L, 5'-TTGAGG-TCTGGAGAGGATGG-3', and E8-R, 5'-AGTGCC-CTTCCTGCTTTACA-3'. The PCR reaction contained standard reagents (AmpliTaq, Perkin-Elmer), with 4% dimethyl sulfoxide, and the amplification conditions were 5 min at 95°C, followed by 35 cycles of 45 s at 95°C, 45 s at 55°C, and 45 s at 72°C, by use of an Omnigene thermocycler (Hybaid). For heteroduplex analysis by CSGE, the PCR products (100–300 ng) were denatured at 95°C, followed by incubation at 68°C for 30–60 min. Samples were run on CSGE gels that contained 40% 99:1 acrylamide:1.4 bis(acryloyl)piperazine, 0.5 \times glycerol-tolerant gel buffer (United States Biochemical), 15% formamide, 10% ethylene glycol, 0.1%

ammonium persulfate, and 0.07% N,N,N',N'-tetramethyl-ethylenediamine (Ganguly et al. 1993). The heteroduplexes were visualized under UV light. The Q243X mutation abolished a restriction-enzyme site for *BsmFI*, which was used for verification of the mutation, by use of the conditions recommended by the manufacturer (New England Biolabs).

Radiation Hybrid Mapping of LAMB3

A 360-bp fragment corresponding to exon 23 of the LAMB3 gene was amplified by use of primers E23-L, 5'-CACTGCTTTAGAGGCCAGAT-3', and E23-R, 5'-GAAAGCATTCCAACCCAATC-3', at the annealing temperature of 57°C under the conditions described above. These oligonucleotide primers were used to analyze the GeneBridge4 radiation hybrid panel (Genome Systems). The resulting data vector (00001 20011 00200 10000 00001 10020 00102 00000 10000 00000 10000 00000 00100 10000 01010 00200 01100 00010 000) was mapped, by use of a LOD score >15, by the RHMapper server, which was accessed on-line at the Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology (Whitehead Institute 1997).

Genotype Analysis

For the genotype analysis of the proband and his parents, two informative intragenic two-allelic polymorphisms within LAMB3, a *BsmFI* polymorphism within exon 4 and a *DdeI* polymorphism within exon 23, were used. The primers for amplification of exon 4 were E4-L, 5'-CCACAGAGGAGATATGCTGG-3', and E4-R, 5'-CCATAACATCCAAGAGACCC-3'. Exon 4 was amplified at the annealing temperature of 55°C, by use of the PCR conditions described above. The primers and amplification conditions for exon 23 are described above. In addition, 17 microsatellite markers outside of the LAMB3 locus spanning the entire chromosome 1, as well as a total of 11 markers representing chromosomes 6, 8, 11, 12, 16, and 17 (obtained from Génethon, except for LAMC2/CA; Aberdam et al. 1994), were used for genotype analysis (table 1). For microsatellite analysis, the sense primer was 5'-end labeled by use of γ -[³³P]-dATP. After PCR amplification, the products were denatured and analyzed on 6% denaturing gels (Sequagel-6, National Diagnostics).

Results

Mutation Detection and Haplotype Analysis of the LAMB3 Locus

Heteroduplex analysis of the mother's DNA demonstrated a band with altered mobility, with the PCR product spanning exon 8 of the LAMB3 gene, whereas the

Table 1

Genotype Analysis of the Family with an H-JEB Patient

MARKER	GENOTYPE, FOR		
	Father	Patient	Mother
D6S428	11	12	22
AFMa082wh9 (chromosome 8)	11	12	21
CHLC.GATA.63F09 (chromosome 11)	32	23	34
D12S90	32	23	31
AFM339XG1 (chromosome 16)	22	22	21
D16S510	21	11	11
D16S509	23	32	21
D17S798	23	31	14
D17S801	13	33	33
CHLC.GATA.41C05 (chromosome 17)	35	52	21
D17S1787	21	13	31

father's and the proband's PCR products showed homoduplex bands only (fig. 1A). Direct nucleotide sequencing revealed that the mother was a heterozygous carrier of a C→T transition at nucleotide position 727 within exon 8 of the LAMB3 gene, resulting in the change of a Gln codon (CAG) to a termination codon (TAG) at amino acid position 243, a mutation designated "Q243X" (fig. 1B). The proband was homozygous for the Q243X mutation, with a T at nucleotide position 727 in both alleles, whereas the proband's father was found to be homozygous for the normal allele, carrying a C in the same position (fig. 1B). This nucleotide substitution abolished the restriction-enzyme site for *BsmFI*, which was used for verification of the mutation (fig. 1C). Restriction-enzyme analysis confirmed that the proband was homozygous for this mutation, whereas the mother was a heterozygous carrier. These analyses further confirmed that the father had two normal alleles in exon 8 (fig. 1), and there was no evidence for other putative mutations in the LAMB3 gene, as was determined by heteroduplex scanning of the father's DNA (data not shown).

To perform haplotype analysis of the LAMB3 locus in the parents and the proband, we first used two informative two-allelic polymorphisms previously characterized within exons 4 (*BsmFI*) and 23 (*DdeI*) (Pulkkinen et al. 1995; Kivirikko et al. 1996) and, therefore, flanking exon 8. The results from studies using these markers clearly showed that the parents were homozygous for different alleles of the LAMB3 gene (fig. 2A). However, in addition to homozygosity for the maternal Q243X mutation, the patient was found to be homozygous for the maternal polymorphic markers on exons 4 and 23, and his genotype showed complete absence of the paternal LAMB3 locus (fig. 2B). Nonpaternity was excluded by use of 11 microsatellite markers from six different

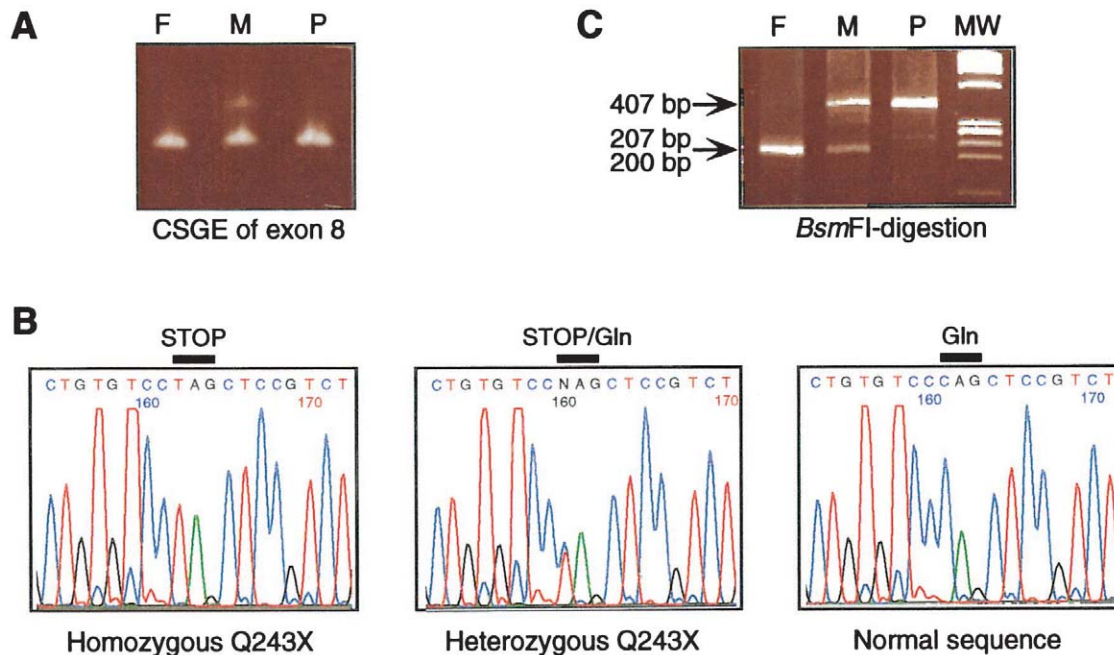


Figure 1 Results of mutation analysis for the family with an H-JEB patient, by CSGE. *A*, Results of heteroduplex analysis. The mother's (lane M) PCR product spanning exon 8 of the LAMB3 gene showed a heteroduplex band, whereas the father's (lane F) and the proband's (lane P) corresponding DNA fragments demonstrated homoduplex bands only. *B*, Results of direct automated nucleotide sequencing. The mother's PCR product (*middle*) demonstrated a heterozygous C→T transition at nucleotide position 727 within exon 8, resulting in substitution of a Gln codon by a termination codon (STOP) at amino acid position 243. The father's PCR product (*right*) was homozygous for the Gln codon, whereas the patient's PCR product (*left*) was homozygous for the termination codon at the same position. *C*, Results of restriction-enzyme analysis. The mutation, designated "Q243X," abolished a restriction-enzyme site for *BsmFI*, which was used for verification of the mutation. The 407-bp PCR product was digested to 207-bp and 200-bp fragments in the normal allele, whereas the mutant allele remained undigested, indicating that the father was homozygous for the normal allele, the proband was homozygous for the mutation, and the mother was a heterozygous carrier of the mutation. MW = HaeIII-digested OX174 molecular weight markers.

chromosomes. The genotypes of the parents and of the proband are shown in table 1.

Radiation Hybrid Mapping of the LAMB3 Gene

In order to localize the LAMB3 gene in relation to other loci on chromosome 1, we used the GeneBridge4 panel with primers E23-L and E23-R (see Patient, Material, and Methods) to produce a human LAMB3-specific PCR marker for radiation hybrid mapping. By use of the RHMMapper program, the data vector was placed 3.98 cR from framework marker WI-5105 on chromosome 1, in the interval bound by polymorphic markers CHLC.GATA87F04 and CHLC.GCT1E07 (fig. 3). Further examination of the Whitehead Institute database (1997) revealed that the previously mapped marker WI-7944 had sequence identity with LAMB3, thus confirming the map location of this gene on chromosome 1q.

Genotype Analysis Using Chromosome 1-Specific Polymorphisms

To elucidate the molecular basis of JEB in this family, we performed additional genotype analyses for chromo-

some 1 in the parents and in the proband. For this purpose, 17 different partly or fully informative microsatellite markers were used (fig. 4). The results demonstrated that the proband was homozygous for a region of maternal chromosome 1 spanning ~35 cM (Gyapay et al. 1994), including, in addition to the mutant LAMB3 locus, the following markers: D1S237, D1S205, D1S245, D1S510, D1S413, D1S412, D1S422, and D1S238 (fig. 4). Other markers telomeric and centromeric from this region, including D1S212, LAMC2/CA, D1S240, D1S229, and D1S439—as well as markers on 1p, including D1S243 and D1S488—showed heterozygosity for both maternal chromosome 1 homologues (fig. 4). D1S189 was homozygous for allele 1, both in the mother and in the proband, again indicating the absence of the paternal chromosome. Some of the markers were only partially informative (fig. 4). On the basis of these results, it was concluded that double recombination had occurred on 1q, placing the recombinational breakpoints between markers D1S238 and D1S240 (centromeric) and between LAMB3 and D1S229 (telomeric), the latter being the closest marker to

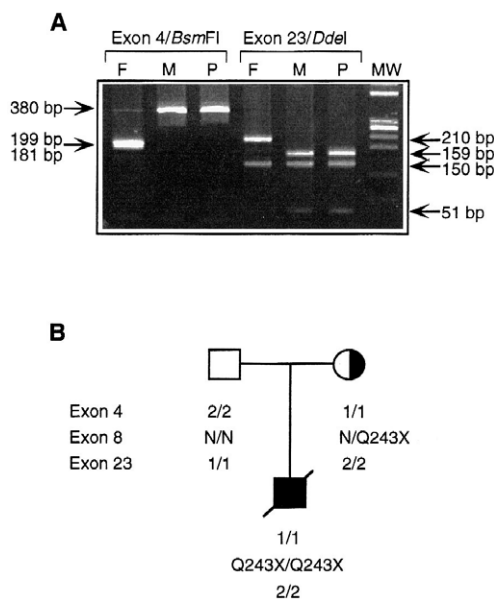


Figure 2 A, Results of haplotype analysis of the LAMB3 gene. Two informative intragenic polymorphisms within exons 4 (*BsmFI*) and 23 (*DdeI*), flanking the mutation in exon 8, were used. PCR products spanning exons 4 and 23 were digested with *BsmFI* and with *DdeI*, respectively. Polymorphic *BsmFI* alleles were 380 bp and 199 bp plus 181 bp, and the corresponding *DdeI* alleles were 210 bp and 159 bp plus 51 bp, whereas the 150-bp band was constant. B, Haplotypes of the parents and the proband, derived from the mutation analysis. *BsmFI* and *DdeI* polymorphisms demonstrated the absence of the paternal LAMB3 allele in the proband.

LAMB3 that showed heterozygosity. On the basis of data from recombinational breakpoints, together with radiation hybrid mapping results, LAMB3 is located between polymorphic markers D1S229 and CHLC.GATA87F04, within an ~3-cM region on 1q (Whitehead Institute 1997). Collectively, these results suggest that the proband has a maternal uniparental primary heterodisomy and a partial isodisomy, also known as “meroisodisomy” (Engel 1993), of chromosome 1.

Discussion

In this study, we describe a patient with H-JEB with a novel pathogenetic mechanism. This patient showed loss of heterozygosity spanning a 35-cM region surrounding the LAMB3 locus on 1q. A deletion was considered unlikely, since karyotyping showed no apparent abnormality in chromosome 1, and a deletion of this magnitude clearly should have resulted in abnormal banding. The results, together with microsatellite analyses of the other regions of chromosome 1, suggested that the H-JEB phenotype in this patient was due to maternal uniparental primary heterodisomy and meroisodisomy of a 35-cM region on chromosome 1q, resulting in re-

duction to homozygosity of the maternally derived non-sense mutation within the LAMB3 gene. The mechanism for this chromosomal aberration could be nondisjunction at the first meiotic division, preceded by recombinational events and followed by either gamete complementation at fertilization or trisomy rescue at postzygotic mitosis (fig. 5). Advanced maternal age has been shown to be associated with nondisjunction in humans, and, in this case, the mother was 35 years of age, thus having an increased risk for nondisjunctional events. The development of the fetus was found to be normal during pregnancy, and the infant, with normal body weight and height, was born with H-JEB.

Our previous studies have demonstrated that the H-JEB phenotype is associated with either homozygous or compound heterozygous PTC mutations in one of the three genes (LAMA3, LAMB3, or LAMC2) encoding the polypeptide subunits of laminin 5 (Uitto et al. 1995; Uitto and Pulkkinen 1996). Irrespective of the mechanism, these PTC mutations predict synthesis of a truncated laminin 5 polypeptide, and, in several cases, reduction of the corresponding mRNA transcript levels, as a result of accelerated mRNA decay, has been demonstrated (Aberdam et al. 1994; Baudoin et al. 1994; Pulk-

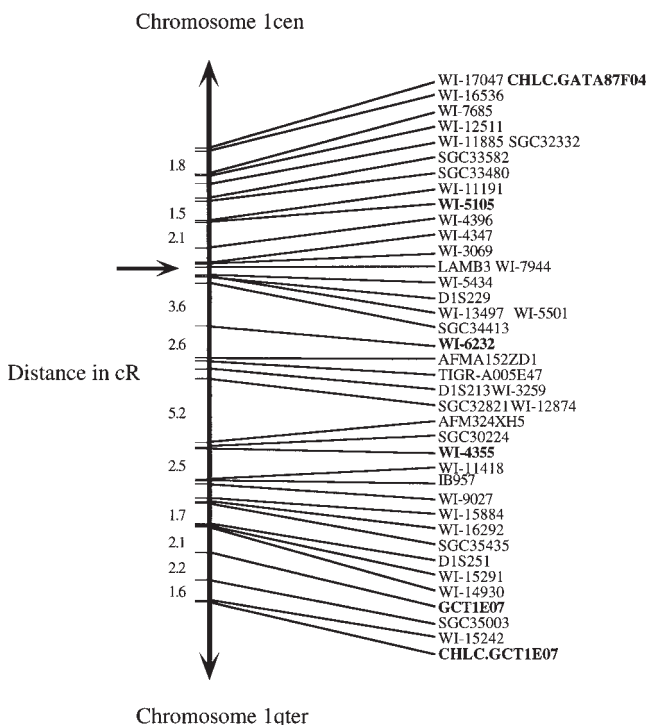


Figure 3 High-resolution radiation hybrid map of the region flanking the LAMB3 locus on 1q. Distances are in centiRays, and the framework markers are indicated in boldface. The gene is flanked by polymorphic framework markers CHLC.GATA87F04 and CHLC.GCT1E07.

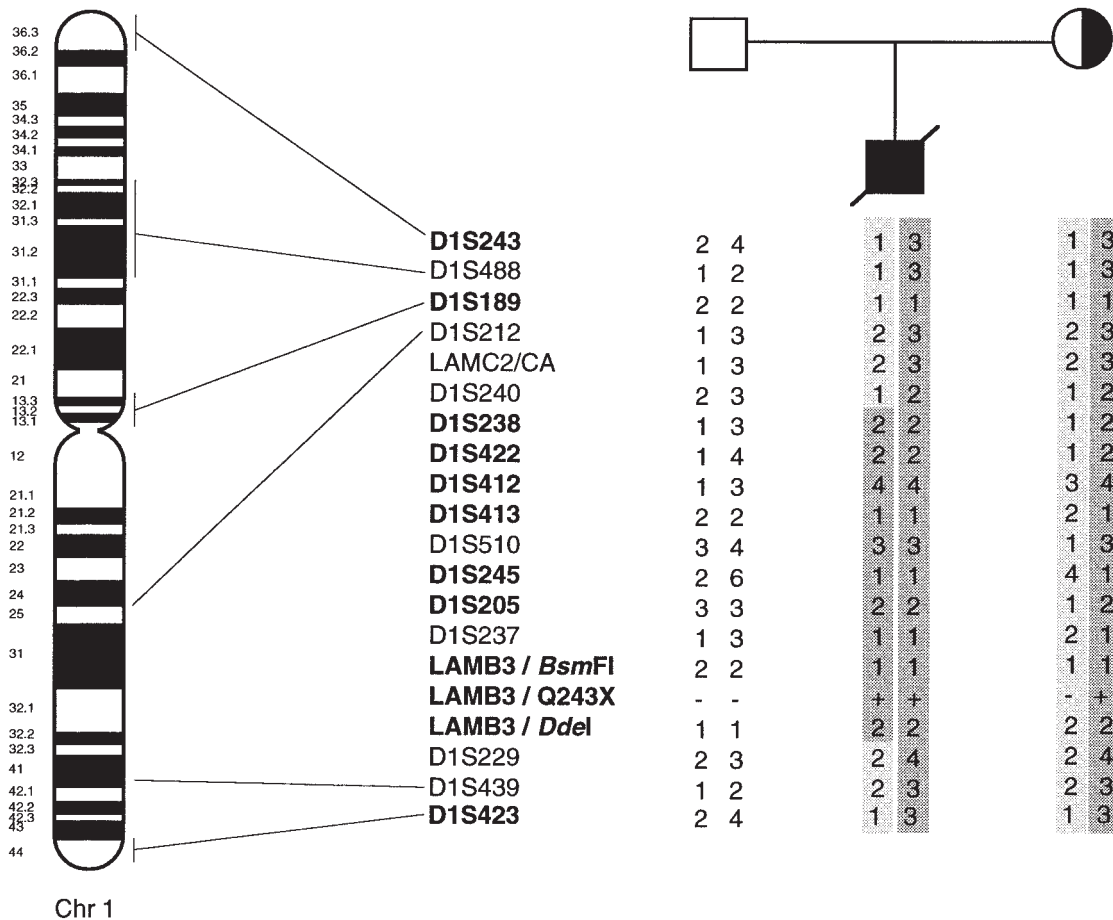


Figure 4 Idiogram of chromosome 1. The polymorphic markers used for genotype analysis in relation to G bands are shown on the *left*. The genotypes of the parents and the proband are shown on the *right*. Different shaded backgrounds demonstrate the alleles on the two maternal chromosome 1 homologues. The markers in boldface are fully informative for maternal UPD of chromosome 1.

kinen et al. 1994; Vailly et al. 1995a, 1995b). As a result of a homozygous or compound heterozygous PTC mutation, no laminin 5 molecules are synthesized, and the assembly of anchoring filament-hemidesmosome complex formation is perturbed. Because of this abnormality, fragility of the skin and other epithelial surfaces ensues, including of those in the gastrointestinal and respiratory tracts. Frequently, as illustrated by the proband in the present study, these clinical manifestations lead to the demise of the affected individual during the early postnatal period.

Demonstration of UPD in the H-JEB patient in this study is the first description of such an event affecting human chromosome 1. On the basis of this study, one could conclude that maternally inherited chromosome 1 has no imprinting effect. In several cases, UPD has been described in association with a normal phenotype, including maternal UPD for chromosomes 2, 13, and 22, as well as paternal UPD for chromosome 21 (Ledbetter and Engel 1995; Bernasconi et al. 1996). Human

chromosome 1 corresponds to homologous regions in mouse chromosomes 1, 3, 4, and 8, and, thus far, there is no evidence for the involvement of these regions in imprinting in mouse (Searle et al. 1994; Ledbetter and Engel 1995). The prime candidate genes for imprinting could be genes crucial for growth and development, such as those encoding different growth factors, oncogenes, peptide hormones, nuclear factors, etc. Human chromosome 1 contains several genes belonging to these categories (OMIM 1997), and rearrangements in chromosome 1 have frequently been described in association with hematologic and other malignancies (Baumgarten et al. 1993; Leonard et al. 1993; Polito et al. 1995; Dierlamm et al. 1996; Michaux et al. 1996; Schlegelberger et al. 1996). It should be noted that, besides characteristic features of H-JEB, the affected infant did not demonstrate any dysmorphic features or developmental abnormalities during the prenatal and early postnatal period. This could reflect the absence of an imprinted gene(s) in maternally derived chromosome 1. Since the affected

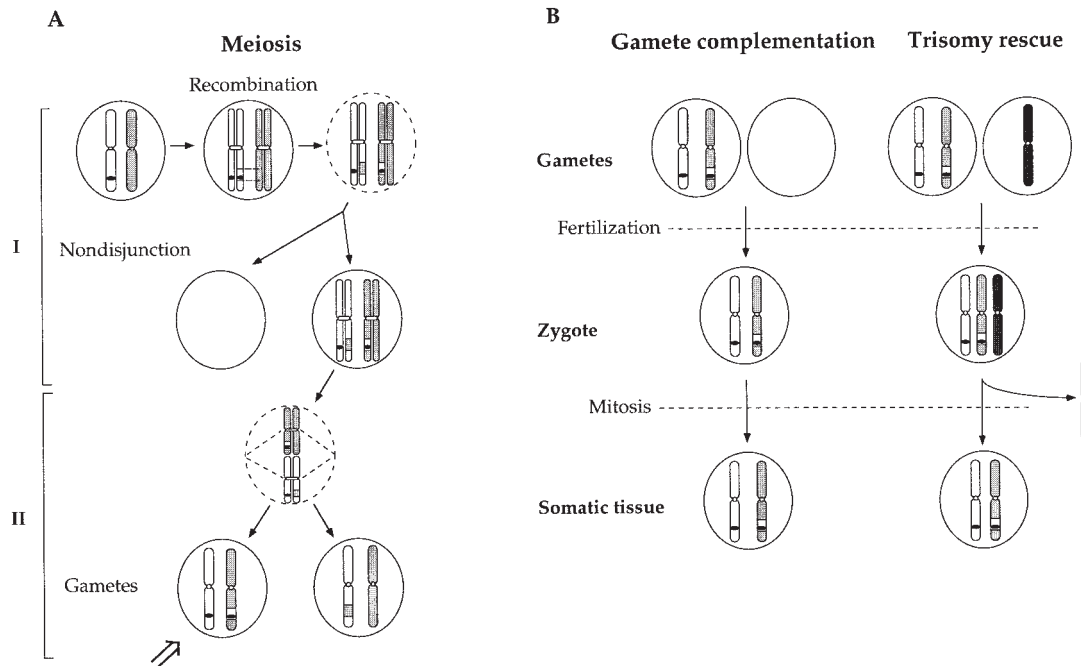


Figure 5 Proposed mechanisms resulting in the H-JEB phenotype in a patient with maternal UPD for chromosome 1. **A**, Proposed mechanism in meiosis. At the prophase stages of meiosis I, the long arms of maternal chromosome 1 homologues containing the mutant (■) and the normal (□) LAMB3 gene undergo recombination, followed by nondisjunction in the first meiotic cell division (I). Chromosome segregation during the second meiotic division (II) results in two disomic gametes, one of which contains two chromosome 1 homologues with a LAMB3 mutation (indicated by the double arrow [↔]). **B**, Two different proposed mechanisms resulting in UPD, by fertilization of the maternal gamete disomic for the chromosome 1 homologues (both of which harbor a LAMB3 mutation). In gamete complementation, the disomic egg cell is fertilized with a paternal nullisomic gamete, resulting in a zygote with UPD for chromosome 1. In trisomy rescue, the maternal gamete is fertilized with a normal paternal gamete containing one copy of chromosome 1 (■), resulting in a trisomic zygote. At the postzygotic mitotic stages, the paternal chromosome homologue is lost from the cells, resulting in maternal UPD for chromosome 1, with a homozygous LAMB3 mutation in somatic cells. Collectively, the maternal LAMB3 mutation was reduced to homozygosity by recombinational and nondisjunctional events, resulting in the H-JEB phenotype.

infant died at the age of 2 mo and since an autopsy was not performed, later developmental milestones could not be evaluated.

The case delineated in this study is 1 among the 61 H-JEB patients thus far studied in our laboratory and demonstrated to have mutations in either LAMB3 or LAMC2, both of which are genes residing in chromosome 1 (Uitto and Pulkkinen 1996; Pulkkinen et al. 1997). This is the first H-JEB case that involved complex chromosomal events, such as uniparental heterodisomy with meroisodisomy.

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