Epidermolysis bullosa (EB) is a group of genetic disorders characterized by fragility of skin and mucosa. The majority of subtypes of EB result from malfunction of anchor proteins of the basal membrane zone (BMZ). Based on the level of blister formation, EB is classified into 4 major forms: EB simplex, junctional EB, dystrophic EB, and Kindler syndrome (Fig. S11).

Junctional EB (JEB) is caused by mutations in genes encoding for components of the lamina lucida, e.g. integrin beta 4 (ITGB4), integrin alpha 6 (ITGA6), laminin-332 genes (LAMA3, LAMB3 and LAMC2), etc. One specific type of JEB with mutations in α6β4 integrin presents with skin fragility in association with pyloric atresia. JEB is inherited only in a recessive manner.

Trisomy 2 mostly results in first trimester pregnancy loss; it is compatible with life exclusively in mosaic states or when restricted to the placental tissues. Common in utero indications of mosaic trisomy 2 are oligohydramnios and poor foetal growth. High trisomic levels in placenta and the foetus can result in abnormal and fatal phenotypes (1). Phenotypes of uniparental disomy of chromosome 2 (UPD2) resulting in trisomy mosaicism have described phenotypes, such as genital hypoplasia, intrauterinal growth retardation (IUGR) and oligohydramnios. Exceptional cases of full UPD2 have been described in healthy individuals (2, 3).

We report here a case of a newborn with JEB resulting from a previously unknown homozygous mutation leading to a premature termination codon in the gene encoding for ITGA6. The loss of heterozygosity occurred in the setting of an exclusive maternal UPD2.

METHODS (see Appendix S1)

CASE REPORT AND RESULTS

A 28-year-old healthy primigravida underwent amniocentesis after ultrasonography performed at 31 weeks of gestation revealed foetal abnormalities (growth retardation, pyloric atresia with polyhydramnios and a fluid-filled stomach, and complex lower-limb anomalies with disproportional long-bone shortening and aplasia of the foot structures).

Amniotic fluid was submitted for cytogenetic analysis and array-based comparative genomic hybridization (CGH) testing. Chromosomal analysis and metaphase fluorescence in situ hybridization (FISH) for chromosome 2 revealed a rare trisomy 2 in 12–15% of all cells in the amniotic fluid with an aberrant 47, XX +2 (2)/46, XX karyotype, confirmed by array-based CGH (Fig. 1A).

Due to foetal growth arrest and polyhydramnios, labour was induced at 37 weeks’ gestation. The newborn was small for gestational age (3 standard deviations (SD) below 1st percentile). Multiple blisters of the skin on the trunk, head, and umbilical cord could be observed, as well as aplasia cutis on all 4 limbs (Fig. 1B). The infant died after 5 days.

Fig. 1. Prenatal and clinical findings. (A) Amniocentesis results at 31 weeks’ gestation. Chromosomal analysis with karyotyping and metaphase fluorescence in situ hybridization (FISH) for chromosome 2 shows trisomy 2 in 12–15% of all cells in the amniotic fluid. Aberrant 47, XX +2 (2)/46, XX karyotype. The comparative genomic hybridization (CGH) array distribution indicates a higher amount of Chr 2 in the patient than in the reference DNA. (B) Clinical images of the patient 1 day after birth with skin aplasia of the left leg, blister on the right side of the head and ear and new blisters following minor trauma (touch) on the torso and left arm.

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Histology revealed a subepidermal blister without inflammation. The basal membrane, visualized by staining for collagen type IV, remained firmly attached to the base of the blister cavity. The ultrastructural sites of the blister formation could be detected within the lamina lucida. There was a decrease in the transmembrane proteins of the hemidesmosomal plate. Immunofluorescence mapping disclosed junctional splitting and loss of immunoreactivity for the integrin α6/β4 (Fig. S2A–E).

Whole exome sequencing (WES) ruled out non-paternity and identified a novel frame-shift mutation in the ITGA6 gene for integrin α6 (patient homozygous, mother heterozygous, and mutation absent from the father) and a missense variant in the COL4A4 gene (patient homozygous, mother heterozygous, and mutation absent from the father). Collagen type IV protein was unaffected both immunohistochemically (Fig. S2B) and on immunofluorescence mapping (Fig. S2E); however, the high pathogenicity of the mutation indicates a possible clinical relevance. COL4A4 mutations have not previously been described in any EB subtype.

The mutation in ITGA6 has not been reported previously. The CADD Phered score of 35 for the ITGA6 mutation places it in the top 0.1% of altering mutations. This novel frame-shift mutation in the ITGA6 gene accounted for the lack of integrinα6 protein in the patient’s skin and was the pivotal feature to diagnose an autosomal recessive inherited junctional epidermolysis bullosa with pyloric atresia (JEB-PA). In confirmation of the results of the immunofluorescence mapping (Fig. S2E) and the WES, Sanger sequencing disclosed a homozygous insertion c.2843_2844dupCG, in exon 22 of the JEB-associated gene ITGA6 (chr2exon22 c.2843_2844dupCG) (Fig. S3A–B). The 2-nucleotide insertion resulted in a frame-shift mutation and a premature stop codon and termination of translation p.Ser949Argfs*15. The mutation was located on chromosome 2, an extra copy of which (trisomy 2) we had identified previously in 12–15% of all cells in the amniotic fluid during the prenatal diagnostic at 31 weeks’ gestation.

At birth, the loss of evidence for persistent trisomy 2, and the clinical manifestation of an autosomal recessive disease in a child born to a wild-type father and a heterozygous mother, suggests a complete chromosomal rescue and unipaternal (maternal or paternal) inheritance of the genes located on the affected chromosome 2.

For an accurate evaluation of the suspected UPD2, postnatal segregation analysis with chromosome 2-specific short tandem repeat (STR) markers was performed. This exhibited exclusive maternal and a lack of any paternal inheritance of chromosome 2 in the newborn, the maternal uniparental isodisomy 2, following the complete chromosomal rescue of trisomy 2, unmasked in a homozygous setting the novel chr2exon22 c.2843_2844dupCG mutation in the ITGA6 gene and resulted in the clinical manifestation of a lethal ITGA6-related JEB-PA.

DISCUSSION

Foetal trisomy can occur due to meiotic non-disjunction events in either parent (4). Trisomy rescue is a rare phenomenon whereby the zygote, with 3 copies of a chromosome, spontaneously loses 1 copy, resulting in a diploid state. Indeed, any copy of the chromosome can be lost, potentially retaining both copies from the faulty gamete, resulting in a UPD (Fig. S4I), identical alleles (isodisomy) of multiple genes, and may be the cause of the clinical manifestation of imprinting or recessive disorders. The rate of segmental UPD is estimated as 1 per 3806 chromosome pairs (0.026%) (5). Whole-chromosome UPD is very rare.

A few cases of JEB have been described with a UPD-based inheritance pattern. Mutations in laminin-332 are associated with the severe subtype of severe generalized (Herlitz) JEB (6). Homozygous mutations in 2 subunits of laminin 332 (LAMB3 and LAMC2) caused by maternal and paternal UPD of chromosome 1 have been reported a few times (7–11). Inheritance of JEB with pyloric atresia through a UPD event has been described (12).

ITGA6 is an important component in embryogenesis with roles in endoderm migration and nervous system development. It forms a heterodimer with ITGB4 (α6β4) in the BMZ. In JEB the ultrastructure of hemidesmosomes is abnormal, and several mutations in ITGA6 and ITGB4 have been reported (13). Some mutations result in less severe phenotypes with longer survival rates; hence the characterization of all ITGA6 and ITGB4 mutations is important to assist patient counselling (14). In the case reported here, the newly detected chr2exon22 c.2843_2844dupCG mutation is strongly indicative of a severe phenotype with a short survival.

ACKNOWLEDGEMENTS

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REFERENCES


Appendix S1.

SUPPLEMENTARY METHODS

Clinical samples

Peripheral blood samples were collected from the patient and both parents. Skin samples were obtained from the patient only. All laboratory investigations were ordered by the patient’s primary neonatologists, after the risks and benefits of testing had been explained to the patient and his family members and written informed consent had been obtained.

Whole exome sequencing and variant confirmation by means of Sanger sequencing

Whole exome sequencing (WES) of genomic DNA extracted from peripheral blood mononuclear cells of the patient and both parents were performed using the SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing Library Protocol Version C0, December 2016 (Agilent Technologies, Santa Clara, CA, USA). Input DNA (200 ng) was fragmented using a Covaris E220 (Covaris Inc., Woburn, MA, USA) to achieve a size of 150-200 bp. End repair, adenylation and adaptor ligation was performed using SureSelectXT Reagents (Agilent Technologies). Exons were hybridized to oligonucleotides from SureSelect Human All Exon V6 (Agilent Technologies) in a 24-h reaction at 65°C. Paired-end 125-bp sequencing was performed on HiSeq4000 Illumina platform (Illumina Inc., San Diego, CA, USA). Mean coverage for patient and parents was >100x.

Bioinformatic analysis was performed as per Genome Analysis Tool Kit v3.5 (GATK) guidelines (Broad Institute, Cambridge, MA, USA). Sequencing output files were in the FASTQ format. These were aligned to the reference genome (hg19) using Burrows-Wheeler Aligner (BWA-MEM). Quality control and recalibration performed with GATK resulted in an output file in BAM format. BAM files were inserted into HaplotypeCaller from Broad Institute to find variations from the reference genome generating a variant called file (VCF). Resulting mutations were annotated using Combined Annotation Dependent Depletion (CADD) (http://cadd.gs.washington.edu/contact) and Variant Effect Predictor (http://www.ensembl.org/info/docs/tools/vep/). Variants that were deemed clinically significant in the JEB-related genes ITGA6 (http://www.ensembl.org/index.html) and ITGB4 located on chromosome 2 were confirmed by Sanger sequencing in the patient and parental samples.

Ultrastructural analysis of the skin

For transmission electron microscopy, the skin biopsies were fixed with Karnovsky fixative (3% paraformaldehyde, 3.6% glutaraldehyde). Post-fixed samples (1% OsO4 containing 1.5% potassium ferrocyanide in aqua bidest, 2 h) were rinsed with distilled water, block stained with uranyl acetate (2% in distilled water), dehydrated in alcohol (stepwise 50–100%), immersed in propyleneoxide, and embedded in glycidyl ether (polymerized 48 h at 60°C; SERVA, Electrophoresis GmbH, Heidelberg, Germany).

Semithin and ultrathin sections were cut with an ultramicrotome (Ultracut, Reichert, Vienna, Austria). Ultra-thin sections (30 nm) were mounted on copper grids and analysed on a Zeiss LIBRA 120 transmission electron microscope (Carl Zeiss, Oberkochen, Germany) operating at 80 kV.

Immunofluorescent antigen mapping

Indirect immunofluorescence staining was performed on 5-μm skin cryosections, which were air-dried and incubated with primary antibodies overnight at 4°C. The primary antibodies used are listed in Table I. The secondary antibodies were Alexa-488 anti-mouse or anti-rabbit IgG (both Invitrogen, Darmstadt, Germany). Nuclei were stained with 4’,6-diamidino-2-phenylindole (DAPI) (Molecular Probes, Temecula, CA, USA). The stained sections were observed with a confocal laser scanning microscope (LSM510, Carl Zeiss, Jena, Germany).

Dermatopathology

For histopathological examination on light microscopy, skin biopsies were fixed with formalin, embedded in paraffin and stained with haematoxylin & eosin (H&E) using standard protocols. For immunohistochemistry (IHC) for collagen IV, the sections were deparaffinized and rehydrated in graded series: X-TRA-Solv 8 (Medite GmbH, Burgdorf Germany), 41-5212-00, 15 min at 68°C; xylo, 5 min room temperature (RT), 100% ethanol, 5 min RT; 96% ethanol, 5 min RT; 80% ethanol, 5 min RT; distilled water, 2 min RT. For antigen retrieval, the slides were heated in a Dako Cytomation Pascal Pressure Cooker (DakoCytomation, Glostrup, Denmark) (115°C) and, endogenous peroxidase activity was then blocked using 3% hydrogen peroxide in distilled water (10 min). Normal goat serum was used to block non-specific epitopes (30 min) and the sections were then incubated with the primary antibody against human collagen IV (Dako Cytomation, #CIV 22, dilution 1:50) as well as the corresponding biotinylated anti-goat IgG secondary antibody (1:100 dilution, 30 min). In accordance with the manufacturer’s protocol (Dako), visualization was achieved via application of streptavidin conjugated to alkaline phosphatase. Additional Mayer’s haematoxylin staining was applied in order to depict the cell nuclei.

Table I. Antibodies used for immunofluorescence mapping

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Supplementary material to article by R. Higgins et al. “Uniparental Disomy of Chromosome 2 Unmasks New ITGA6 Recessive Mutation and Results in a Lethal Junctional Epidermolysis Bullosa in a Newborn”

Fig. S1. Major forms of inherited epidermolysis bullosa (EB). (A) Structure of normal human skin. (B–D) Site of blister formation in EB subtypes: (b) EB simplex, (c) junctional EB, (d) dystrophic EB.

Fig. S2. Morphological and ultrastructural findings. (A) Conventional histology with subepidermal blister formation without signs of inflammation. (B) Immunohistochemical staining for collagen type IV, forming the base of the subepidermal blister cavity. (C) Electron microscopy of a basal keratinocyte from the patient with decrease in the transmembrane proteins of the hemidesmosomal plate of the patient. (D) Interconnected plaques with intermediate filaments (yellow), lamina densa (green), integrins (red), laminin335 fibrils (dark blue), collagen IV (light blue) in healthy control skin. (E) Immunofluorescent antigen mapping and detection of a large junctional split (star) and reduction in integrin α6 and loss of β4 expression, compared with healthy control (F).
Supplementary material to article by R. Higgins et al. “Uniparental Disomy of Chromosome 2 Unmasks New ITGA6 Recessive Mutation and Results in a Lethal Junctional Epidermolysis Bullosa in a Newborn”

Fig. S3. Genetic analysis. (A) Results from whole exome sequencing identifying the ITGA6 mutation in exon 22. (B) Confirmation by Sanger sequencing and identification of a stop codon mutation c.2843_2844dupCGp.Ser949Argfs*15. The patient was homozygous for T to C mutation. The mother was heterozygous, and the father was not a carrier. (C) The ITAG6 pedigree, with squares representing male members, circles female members, shading members with autosomal recessive JEB, and the dot indicating the silent mutation carrier.

Fig. S4. Mechanism of uniparental disomy occurrence. (A) Chromosomal pattern in parents. (B) The process of meiotic non-disjunction resulting in a trisomy. In most cases this will result in spontaneous abortion or miscarriage. (C) The process of trisomic rescue, where 1 copy of the chromosome is expelled to form a diploid structure. In most cases this results in a normal embryo, but in 30% of cases a uniparental disomy will occur.