ELECTRONIC LETTER

Selective disruption of muscle and brain-specific *BPAG1* isoforms in a girl with a 6;15 translocation, cognitive and motor delay, and tracheo-oesophageal atresia

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The protein *BPAG1* (MIM: 113810, 600088, <http://www.ncbi.nlm.nih.gov/Omim/>) Dystonin is a 230 kDa hemidesmosomal protein belonging to the plakin family, originally identified as one of the major autoantigens of bullous pemphigoid (BP), an autoimmune subepidermal skin blistering disease.¹ Mutations in the Dystonin gene result in sensory neuron degeneration in the mutant mouse (dystonia musculorum (dt/dt)).²⁻⁴

The complex organisation of the gene has gradually emerged, initially with the discovery of neuronal isoforms with different NH2-terminal sequences,^{3 5 6} and more recently with the demonstration of the existence of much longer brain- and muscle-specific isoforms with a complex COOH-terminal organisation.^{7 8}

Although a complete understanding of the variety of *BPAG1* isoforms has not been reached yet, a general picture of the organisation of the gene is now available. The epithelial isoform (*BPAG1e*) is made of a coiled-coil (CC) rod domain flanked by an NH2-terminal head domain called the plakin domain,⁹ and by a COOH-terminal tail domain made of plectin repeats, capable of binding intermediate filaments (IF) and for this reason called the IF-binding domain (IFBD).⁹ The plakin domain binds BP180, the other major autoantigen in BP.¹⁰

The brain-specific isoform (*BPAG1a*) has an NH2-terminal actin-binding domain (ABD) made of two calponin homology domains,^{3 5} a plakin domain, a series of spectrin repeats and a COOH-terminal containing two EF-hand calcium-binding motifs, and a growth arrest-specific 2 (GAS2) microtubule-binding domain.^{7 11} An alternative NH2-terminal isoform produces a protein with a single calponin-homology domain, destroying the actin-binding activity and at the same time uncovering microtubule-binding activity.⁶

The muscle-specific isoform (*BPAG1b*) has a structure very similar to the brain isoform, but includes an additional central IFBD, encoded by a single large exon.^{7 8} The binding specificity of this domain has not been tested yet.

BPAG1/Dystonin is a member of a family of giant cytoskeletal proteins showing properties of both the spectrin and plakin superfamilies, named spectraplakins.¹² To this family also belong the mammalian MACF1^{6 11 13 14} and the *Drosophila* short stop (*shot*) genes.¹⁵⁻²⁰

Since spectraplakins are able to interact with all three cytoskeletal components (IF, microtubules, actin), their functions may include: crosslinking cytoskeletal filaments; linking the cytoskeleton to plasma membranes; organising the interaction between the cortical cytoskeleton and plasma membrane proteins to generate membrane subdomains; and acting as scaffolding proteins to recruit signalling proteins to sites of cytoskeletal activity (reviewed in Roper *et al*, 2002¹²).

The obvious importance of each of these functions for the cell makes these genes good candidates for involvement in

Key points

- We report on a female child with oesophageal atresia (EA) and psychomotor retardation associated with a "de novo" reciprocal translocation t(6;15)(p11.2;p12). The patient presents a non-progressive encephalopathy, severe motor and mental retardation, and delayed visual maturation. The 6p breakpoint falls within the *BPAG1* gene selectively interrupting the two brain- and muscle-specific isoforms.
- *BPAG1* codes for a hemidesmosomal protein belonging to the plakin family, originally identified as one of the major autoantigens of bullous pemphigoid (BP). Homozygous *BPAG1* knock-out mice show neurodegenerative disease and develop progressive ataxia due to the degeneration of the sensory neurons. No mutations for *BPAG1* have been reported so far in humans.
- We discuss the possibility that *BPAG1* haploinsufficiency or abnormal expression may determine the phenotypical abnormalities of our patient.

different genetic pathologies. Indeed, *Drosophila* *shot* mutations are embryonic lethal when homozygous, generating defects in epidermal integrity, epidermal muscle attachments, axon outgrowth, dendritic branching, neuromuscular junctions, and sense organ development, and anastomosis of the tracheal branches.^{16 18 19 21-23}

Mice homozygous for *BPAG1* mutations suffer progressive degeneration of the sensory neurons.⁴ Defects in the function of Schwann cells²⁴ and muscles^{25 26} have also been reported. In addition, a knockout mutation eliminating the plakin domain-encoding exons (probably generating a null phenotype) has a skin blistering phenotype similar to the one found in the human disease epidermolysis bullosa simplex.⁴ A similar phenotype, epidermolysis bullosa simplex associated with muscular dystrophy, is caused by mutations in the

Abbreviations: ABD, actin-binding domain; BP, bullous pemphigoid; CC, coiled-coil; DMEM, Dulbecco's modified Eagle medium; EA, oesophageal atresia; EBV, Epstein-Barr virus; FBS, fetal bovine serum; FISH, fluorescent in situ hybridisation; FSHD, facioscapulohumeral muscular dystrophy; HAT, hypoxanthine-aminopterin-thymidine; IF, intermediate filaments; IFBD, IF-binding domain; LCL, lymphoblastoid cell line; ODED, oculo-digito-oesophageal-duodenal; PA-JEB, junctional epidermolysis bullosa with pyloric atresia; PBL, peripheral blood lymphocytes; RPMI medium, Roswell Park Memorial Institute medium

human plectin gene.^{27 28} No human disease associated with alterations in the *BPAG1* gene has been reported so far.

We describe a girl with a balanced t(6;15) translocation showing profound cognitive and motor delay and tracheo-oesophageal atresia. The abnormal phenotype of the probanda is presumably associated with the breakpoint on chromosome 6 since no protein-coding genes have been found on the short arm of chromosome 15. The translocation's breakpoint interrupts the *BPAG1* gene just downstream of the end of the *BPAG1*e isoform coding region, selectively disrupting the *BPAG1*a and b isoforms.

CASE REPORT

The proband, now 4 years old, was the first child born to African healthy unrelated parents. During pregnancy the mother presented threatened abortion and polydramnios. Antenatal ultrasound screening revealed oesophageal atresia and a single umbilical artery. The baby was delivered by elective caesarean section at the 35th week of gestational age. Birth weight was 2200 g (25th–50th centile). The diagnosis of oesophageal atresia was confirmed due to failure to pass a nasogastric tube into the stomach (the tube stopped 9 cm from the gingival margin) and through radiological studies of the upper oesophageal pouch (projection of end at level of D1–D2).

The absence of other congenital anomalies lead to classification of the newborn under the group A risk of the Waterston classification.²⁹ Surgical correction was performed in the 1st day of life. A right side transpleural thoracotomy was carried out, the distal tracheo-oesophageal fistula was closed, and a primary oesophageal anastomosis was performed. The anomaly was classified as a long gap oesophageal atresia with distal tracheo-oesophageal fistula (type 3 of the Roberts classification³⁰).

Postoperative course was complicated by gastro-oesophageal reflux unresponsive to medical therapy. A subsequent oesophago-gastro-duodenal endoscopy demonstrated low grade oesophagitis and an accessory pancreatic duct opening in the stomach. A fundoplication surgical procedure was carried out when the baby was 1 year old due to the presence of severe gastro-oesophageal reflux. At 3 year follow up the patient can eat without problems.

From a neurological point of view, the child presented a non-progressive encephalopathy, characterised by mild pyramidal and cerebellar signs (hyperreflexia, muscle hypotonia, and truncal ataxia), severe motor and mental retardation, and delayed visual maturation. During the 1st year of life prominent neurological signs were severe visual impairment and motor and cognitive delay, associated with mild muscle hypotonia and increased tendon reflex. At this time ophthalmologic examination was normal but visual evoked potentials were delayed and reduced in amplitude; brain MRI documented mild cerebral atrophy, without other malformations or abnormalities of visual pathways or of the posterior fossa. EEG revealed bursts of slow waves during wake and sleep; brainstem evoked potentials were normal; echocardiography was normal. The child's psychomotor development has been characterised by very slow improvement of motor abilities (independent walking was achieved at 30 months), and by the acquisition of minimal cognitive and linguistic abilities. Basic visual functions (visual acuity, fixation, and visual pursuit) have progressively improved, while visual evoked potentials normalised by the age of 12 months, supporting the diagnosis of delayed visual maturation. The child is now 4 years old, and on neurological examination presents mild hyperreflexia, gross motor incoordination and gait ataxia, severe visual inattention, and mildly reduced visual acuity. Her mental age, measured using the Griffiths scale,³¹ is less than 1 year (severe mental retardation): she is

able to recognise familiar persons, sometimes smiles in response to smiles, but does not usually attempt to interact with others. Some utterances are present, but she does not usually use words in a meaningful way and her expressive language is limited to a pre-speech level (crying and vocalising but no use of gesture or imitative behaviour). She shows no interest in toys. EEG, and visual and brainstem evoked potentials are within normal limits. The child has no skin abnormalities, and no problems with wound healing were reported.

METHODS

Cytogenetic characterisation

Routine cytogenetic analysis was performed on the proband's blood using standard high-resolution techniques³² and DA-DAPI staining. Fluorescent in situ hybridisation (FISH) was done with a commercial (Vysis) mixture of probes including CEP 15 (D15Z1: 15p), SNRPN (15q11–q13), and PML (15q22) according to the supplier's instructions and with PAC (P1 artificial chromosome) RP1-61B2 containing the gene *BPAG1* (6p12.1: NCBI Map Viewer, <http://www.ncbi.nlm.nih.gov>; Santa Cruz Human Genome Browser, <http://genome.cse.ucsc.edu/cgi-bin/hgGateway/>; The Wellcome Trust Sanger Institute, <http://www.sanger.ac.uk>). The PAC was labelled with biotin-dUTP (Vector Laboratories, Burlingame, CA) using standard nick translation and visualised with FITC-avidin (Vector) and the chromosomes were counterstained with DAPI (Sigma Aldrich, Milan, Italy).

Parents' karyotypes were analysed in G-banding.

Generation of somatic cell hybrids

The HPRT-negative RJK88 Chinese hamster cell line was maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco). A lymphoblastoid cell line (LCL) was generated from the proband's peripheral blood lymphocytes (PBL) with Epstein-Barr virus (EBV), and maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% FBS (Gibco). RJK88 cells were seeded at a concentration of 2×10^7 cells per 100 mm tissue culture dish. After 24 h, cells were washed with DMEM without serum, and 5×10^7 LCL cells were added in 5 ml of DMEM without serum containing 125 μ l of a 1 mg/ml stock of phytohaemagglutinin-P (Sigma). Dishes were incubated at 37°C for 15 min. The media were gently aspirated, and 2 ml of 50% polyethylene glycol (1000 MW; Sigma) was layered over each dish. After 1 min treatment, cells were washed three times with DMEM and incubated in 10 ml of serum-free DMEM at 37°C for 30 min. Cells were allowed to recover overnight in 10 ml DMEM, 10% FBS. The next day cells from each 100 mm dish were trypsinised and seeded 1:5 into fresh 100 mm dishes containing selective media composed of DMEM with 10% FBS and a 1×solution of hypoxanthine-aminopterin-thymidine (HAT; Sigma). Cells were fed fresh selection media every 2–3 days. After 2–3 weeks, individual colonies were transferred to a 24 well plate and expanded in duplicate 6 well plates. One well was used for genomic DNA isolation and genotyping, while the other was used for freezing of the clone. Clones were maintained in HAT media throughout the experiment.

DNA extraction and genotyping

Genomic DNA of the proband and her mother was extracted from venous blood using standard protocols. DNA from hybrid clones was extracted with Isoquick (Orca Research, Bothell, WA). Genotyping of polymorphic loci was performed by amplification with primers labelled with fluorescent probes (ABI 5-Fam, Hex, and Tet) followed by analysis on

a ABI 310 Genetic Analyzer (Applied Biosystems). Non-polymorphic loci were assayed by electrophoresis on agarose gels. The UCSC Genome Browser (accessed June 2002) maps and sequence were used as references. Amplifications were performed with Taq Gold (Applied Biosystems) using standard protocols.

Inverse PCR was performed on RsaI cut, ligated (in 1 ml volume to facilitate self-ligation of individual fragments) genomic DNA, using nested sets of primers at different positions. Amplified fragments were isolated on a 1% agarose/TAE gel, purified with Qiaex II, and sequenced with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were performed with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and run on an ABI Prism 310 Genetic Analyzer.

RNA extraction and expression analysis

Total RNA was extracted from tissues and cell lines with Eurozol (Euroclone) following the manufacturer's protocols, and human tissues total RNA was purchased from Clontech. cDNA synthesis was performed with Ready-To-Go You-Prime First strand beads (Amersham) and random hexamers. cDNA amplifications were performed in 25 µl reactions, using JumpStart Red ACCUTaq LA DNA polymerase (Sigma) and the following protocol: 1 min at 96°C, 30 cycles of 30 min at 94°C/2 min at 68°C, 5 min final elongation time. The primers for allele-specific expression analysis were hBPAG1 For (5' TCAAGTTCATGGACCTAAGGACT 3'), SNP-E35/1F (5' CCGCTGAAAGAGAACTGGA 3'), and SNP-E35/1R (5' CTGGAGGGCATTAAAGTTCATA 3'); the primers for isoforms-specific amplification were hBPAG1 For, hBPAG1e Rev (5' TTTCTCCAAGTATTGCGAAAATTCAG 3'), hBPAG1 a Rev (5' AATTAGGCGGTTTTTCAGTTTGGGTAAGA 3'), and hBPAG1 b Rev (5' ATGAGGCCTCTTAAACTGCTTGAAAG 3'). Control human G3PDH primers were from Clontech. The cDNA for 5' RACE was synthesised and amplified using a Smart RACE cDNA amplification kit (Clontech).

RESULTS

Cytogenetic and molecular characterisation

Cytogenetic analysis of the probanda revealed a reciprocal translocation interpreted as t(6;15)(p11.2;p12) (fig 1A). DA-DAPI staining showed that the 15p DA-DAPI positive region was still present on the der(15) (data not shown). FISH analysis confirmed the presence of satellite III DNA on the der(15) (fig 1B). Parents' karyotypes were normal.

We screened 80 hybrid clones and found two containing a normal chromosome 6, two with a der(6), and one with a der(15). Genotyping the proband, her mother, and representative hybrid clones for polymorphic markers on both sides of the breakpoint (table 1) demonstrated that the translocation had taken place on the paternal chromosome. FISH analysis confirmed that the chromosome 6 breakpoint was within PAC RP1-61B2, which gives three signals localised at the normal chromosome 6 and at both derivatives, respectively (fig 1C).

Based on the UCSC map, we typed the informative hybrid clones for chromosome 6 markers and eventually restricted the breakpoint location to a 400 bp region about 2 kb downstream of the polyadenylation site of the BPAG1e isoform (fig 2A). The breakpoint was cloned by inverse PCR from the proband's genomic DNA (fig 2B). There is no known feature on chromosome 6 that could be directly responsible for the translocation, except for an Alu repeat immediately flanking the breakpoint. All attempts to obtain additional chromosome 15 breakpoint sequence information by inverse PCR with other restriction enzymes failed.

The breakpoint does not disrupt the portion of *BPAG1* coding for the epithelial isoform, but selectively interrupts the two longer isoforms recently demonstrated in the mouse.⁷

We sought to analyse whether the translocation also affects the expression of the epithelial isoform of the gene and searched for informative polymorphisms in its coding region. We found a heterozygous G/A polymorphism (dbSNP rs# cluster id: rs2230862, <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp>) in the first of the two exons

Figure 1 Cytoogenetics and FISH results of the proband's lymphocytes. (A) Cut-out (left) and ideogram (right) of the normal (on the left) and the derivative (on the right) chromosomes 6 and 15. (B) FISH with probes D15Z1 (green signals), SNRPN (red signals close to the green signals), and PML (red distal signals) shows that the chromosome 15 breakpoint is on the short arm, distal to satellite III (D15Z1). (C) FISH with PAC RP1-61B2 demonstrates that the chromosome 6 breakpoint is within this clone. In B and C arrowheads indicate the normal chromosome 6 (green arrowhead) and the der(6) (white arrowhead), while arrows indicate the normal chromosome 15 (green arrow) and the der(15) (white arrow).

Table 1 Molecular genotyping of the proband and her mother for informative chromosome 6 polymorphisms

Locus	Proband	Mother	Chr. 6 clone	der(15) clone	der(6) clone
D6S459	89/91	89/97	89	91	
D6S452	262/266	266/268	266	262	
D6S455	149/160	145/149	149		160
D6S430	228/232	232	232		228

Chr., chromosome. The father's DNA was not available. Typing of representative fusion hybrid clones is also shown.

coding for the rod domain of the BPAG1e isoform, and determined the genotype of the alleles on the normal chromosome 6 (it carries the G allele) and the der(6) (it carries the A allele). Then we analysed the expression of the BPAG1e isoform in the proband's LCL cells and hair roots (fig 3). We also analysed some heterozygous control subjects. The proband's LCL amplifies BPAG1e cDNA about ten times more than the control LCL cells (fig 3A) and expresses exclusively the der(6) allele (fig 3B); hair roots express both alleles, although the allelic ratio varied between experiments, probably due to the small amount of RNA available.

Next, we analysed the expression of *BPAG1* isoforms in a number of human tissues (fig 4A) using isoform-specific PCR (as in Leung 2001, modified for the human gene⁷). Sequence analysis of isoforms-specific amplification products and 5'RACE experiments (not shown) demonstrated that the alternative splicing pattern seen in the mouse *BPAG1* gene is maintained in humans. BPAG1e is expressed in prostate and trachea; BPAG1a is expressed predominantly in brain and cerebellum, but also in prostate, testis, and trachea; BPAG1b is the only isoform found in lung and skeletal muscle, but is also expressed in brain, prostate, testis, and trachea. We also

Figure 2 (A) *BPAG1* gene and transcript's partial structure. The *BPAG1* gene is shown in cen-tel orientation. Transcription of the *BPAG1* gene is left to right. Only the portion of the gene close to the breakpoint is shown. The gene structure was obtained by combining the UCSC map data (based mainly on Okumura *et al* 2001⁸) and the mouse gene structure elucidated by Leung *et al* (2001)⁷. The protein domains corresponding to each gene region are shown on top. The location of all primers used for allele-specific expression analysis is indicated by blue arrowheads, while the position of the informative SNP is indicated by an orange bar in the CC rod domain exon; the primers used for isoform-specific expression analysis are represented by orange arrowheads. The breakpoint's location is indicated by a vertical arrow. (B) Breakpoint sequences. Chromosome 15-derived sequences are underlined; an AluJb repeat close to the breakpoint location on chromosome 6 is shown in lowercase letters; there is no ATTTC sequence on either derivative chromosome.

Figure 3 (A) Expression of BPAG1e in hair and LCL samples. BPAG1e and G3PDH amplifications were performed as described in Methods. C1–C2, control subjects; M, molecular weight marker X (Roche Diagnostics, Monza, Italy); P, proband. (B) Allele-specific expression of BPAG1e in tissues from proband and control (C1). All sequences show the coding strand of the gene. Genomic amplification was performed with primers SNP-E35/1F and SNP-E35/1R, cDNA amplification with hBPAG1 For and SNP-E35/1R.

analysed the expression of *BPAG1* isoforms in oesophagus samples from normal controls. All *BPAG1* isoforms are expressed in normal oesophagus, while the oesophageal epithelium expresses mainly the e form (fig 4B).

DISCUSSION

The child we studied carries a de novo balanced translocation associated with oesophageal atresia and cognitive and motor delays.

Reciprocal translocations associated with Mendelian phenotypes have been instrumental in the identification of many disease genes.³³ Breakage of one or both chromosomes can cause loss of function phenotypes by disrupting the coding sequence of dosage-sensitive genes (see for example elastin and supravalvular aortic stenosis³⁴) or by separating the genes from the nearby regulatory sequences (see for example *SOX9* and campomelic dwarfism³⁵). Alternatively,

the translocation may cause gain of function phenotypes by switching the regulatory sequences from one gene to another, thus leading to inappropriate expression, or by producing a chimeric gene coding for a new chimeric protein. This is a common mechanism leading to tumour processes in leukaemias, lymphomas, and sarcomas (for a review see Rego and Pandolfi, 2002³⁶). In other cases one of the breakpoints of the chromosomes involved in the reciprocal translocation lose submicroscopic portions containing dosage-sensitive gene(s) (see for example *SOX2* and anophthalmia³⁷). In our case, the abnormal phenotype of the probanda was presumably associated with the breakpoint on chromosome 6 since no protein-coding genes are present on the short arm of chromosome 15. The p-arms of human acrocentric chromosomes contain organised arrays of beta-satellite and D4Z4 repeats and rDNA. The beta-satellite and D4Z4 repeats appear at the distal end of the arm, while rDNA and D4Z4 repeats are

Figure 4 Expression of *BPAG1* isoforms in human tissues. (A) 1: Adrenal gland; 2: bone marrow; 3: brain, cerebellum; 4: brain (whole); 5: heart; 6: kidney; 7: liver; 8: lung; 9: placenta; 10: prostate; 11: salivary gland; 12: skeletal muscle; 13: spleen; 14: testis; 15: thymus; 16: thyroid gland; 17: trachea; 18: uterus; 19: colon; 20: small intestine. Molecular weight markers (M) are always Marker V for *BPAG1* isoforms and Marker X for G3PDH (Roche, Diagnostics, Monza, Italy). (B) Expression in oesophageal lining (adult female, biopsy) and whole oesophagus.

interspersed at a more proximal location, corresponding to the p12.^{38,39} The finding that at the chromosome 6 breakpoint the *BPAG1* gene was interrupted resulting in the disruption of both brain- and muscle-specific isoforms suggests that *BPAG1* haploinsufficiency may cause the patient's clinical symptoms, or a truncated form of BPAG1 a/b could be expressed in tissues and interfere with the activity of the wild type protein, leading to a dominant negative phenotype. Of course, we cannot exclude that the maternal *BPAG1* allele carries a mutation, leading to a recessive phenotype, or that the proband's phenotype is unrelated to the translocation.

The genomic organisation of the human and mouse *BPAG1* genes is very similar,^{6,8} and our expression data on the three isoforms closely parallel those obtained in the mouse.

Dystonia musculorum (dt) is a recessively inherited neurodegenerative disease in mice, first visible between 7 and 10 days after birth. It develops as a progressive ataxia, due to the degeneration of the sensory neurons in the CNS.^{2,4} The animals also show intrinsic muscle weakness and instability of skeletal muscle cytoarchitecture, as well as reduced resistance of stratified epithelia to mechanical stress.^{25,26} In the dt/dt mouse, lack of *BPAG1* probably induces a slow, initially subtle disorganisation of the developing neuronal cytoskeleton, eventually leading to disruption of the normal axioplasmic flow, resulting in focal swelling and neurodegeneration. Heterozygous mice apparently do not show any impairment. However, Bernier and colleagues⁴⁰ analysed the effects of dystonin mutations on Schwann cell structure and myelination of nerve fibres, and observed structural abnormalities and myelination defects. They also noted a gene dosage effect with heterozygous animals presenting hypo- and hyper-myelinated peripheral nerves.

The lack of one copy of *BPAG1* or the presence of truncated forms of the protein could produce a subtler effect in humans

on the same structures, perhaps leading to a partial disorganisation of the sensory and motor circuits. In our patient, the translocation is associated with a profound delay in the acquisition of cognitive and motor skills, as well as in visual maturation. No effects on epithelial tissues are visible, in agreement with the finding that the *BPAG1* epithelial isoform is not disrupted by the translocation. When we tested allele-specific expression of the *BPAG1e* isoform in order to assess whether any position effect might prevent transcription of the der(6) allele, we could show that both alleles are expressed in the proband's hair roots. Unexpectedly, proband's LCL generated very high levels of *BPAG1e* amplification, apparently all from the der(6) allele. It is tempting to speculate that the unusual level of expression in LCL may be related to the presence on the der(6) chromosome of sequences from 15p. Our cytogenetic analysis has demonstrated that the 15p breakpoint is located in the band containing rDNA and D4Z4 repeats. D4Z4 repeats are involved in the genesis of facioscapulohumeral muscular dystrophy (FSHD), and numerous studies have demonstrated their role in chromatin structure control (reviewed in Bickmore and van der Maarel, 2003⁴¹), while rDNA localises to the nucleolus in interphase cells.

To our knowledge, the effects of repeated sequences from the human acrocentric chromosome short arms on the expression of a nearby gene have not been analysed in a naturally occurring translocation or in a reporter gene construct.

Could the translocation also have caused or facilitated oesophageal atresia (EA) in the probanda? Congenital EA occurs in about three of 10 000 live births^{42,43} as a result of faulty development of the primitive foregut. In over 90% of such cases tracheo-oesophageal fistula is also present. Most cases are sporadic, but familial cases do exist (reviewed in Van Staey *et al*, 1984⁴⁴). One dominant form of EA, the

oculo-digito-oesophageal-duodenal (ODED) syndrome, has been mapped to chromosome 2p23–24.⁴⁵ Non-syndromic EA is considered to be a multifactorial trait whose pathogenesis and causation are ill defined. The recurrence of EA in some families suggests a contribution of genetic factors. The association of EA with cognitive and motor delay in the absence of other congenital anomalies has not been specifically described.

We have shown that all three *BPAG1* isoforms are expressed in the human adult trachea. *BPAG1e* is expressed in the epithelial layer of the oesophagus, while the whole tissue expresses all three isoforms.

BPAG1 expression in the developing digestive tract has never been analysed, and neither *dt/dt* nor *BPAG1* KO mice show any obvious defects. On the other hand, there are data indicating a role for spectraplakins in organising morphogenetic events, such as the requirement for shot in the anastomosis of the developing tracheal tubules in *Drosophila* embryos,⁴⁶ or the alteration of F-actin organisation by plectin.⁴⁷

Genetic defects affecting the integrity of the basement membrane and hemidesmosomes can involve the digestive tract. This is the case in junctional epidermolysis bullosa with pyloric atresia (PA-JEB, MIM: 226730), an autosomal recessive disorder that can be caused by mutations in the integrin-beta-4 gene (*ITGB4*; MIM: 147557) and the integrin-alpha-6 gene (*ITGA6*; MIM: 147556). Association with oesophageal atresia has been reported in very few cases.^{48–49}

In view of the size of the *BPAG1* gene and its exceptionally complex structure, mutational analysis in subjects with oesophageal atresia is hardly feasible, but immunohistochemical analysis of surgically removed tissues may be an option.

This study describes the first human subject with a specific disruption of the brain- and muscle-specific isoforms of *BPAG1*. The clinical picture of the patient is not easy to compare with the phenotype of the *BPAG1*-deficient mice, particularly in the absence of biochemical and histological data. Nevertheless, the pattern of expression of *BPAG1* supports the hypothesis that the patient's symptoms, and particularly her profound neurological impairment, are a direct consequence of the disruption of *BPAG1* brain and muscle isoforms, leading to insufficient or inappropriate production of protein during embryogenesis.

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