ELECTRONIC LETTER

Patient with bilateral periventricular nodular heterotopia and polymicrogyria with apparently balanced reciprocal translocation t(1;6)(p12;p12.2) that interrupts the mannosidase alpha, class 1A, and glutathione S-transferase A2 genes

E P Leeflang, S E Marsh, E Parrini, F Moro, D Pilz, W B Dobyns, R Guerrini, J W Wheless, J G Gleeson

J Med Genet 2003;40:e128 (<http://www.jmedgenet.com/cgi/content/full/40/12/e128>)

Polymicrogyria (PMG) is a cortical development defect that results in an irregular brain surface, with multiple, small, partly fused gyri separated by shallow sulci. The perisylvian form is the most common pattern of PMG seen on magnetic resonance imaging scans of the brain. Bilateral perisylvian PMG (BPP) often is accompanied by mild mental retardation, epilepsy, and pseudobulbar palsy, and this results in problems with expressive speech and feeding.¹

Bilateral periventricular nodular heterotopia (BPNH) is a neuronal migration disorder, in which neurons that fail to migrate into the developing cortex remain on the ventricular surface and form nodules that line the lateral ventricles.² It can be inherited as an X linked dominant trait.³ Female patients with BPNH are of normal intelligence, have seizures, and may have complications in the vascular system, including patent ductus arteriosus and coagulopathy. Few male patients with BPNH have been reported^{4,5}; however, most male fetuses with BPNH are not viable. Male patients with BPNH may present with mental retardation, epilepsy, and other congenital anomalies, including cerebellar hypoplasia and syndactyly. Mutations in *filamin A* (*FLNA*, also known as *filamin 1*, OMIM 300017) on Xq28 were found to be causative for BPNH.⁶ Many patients with BPNH do not harbor mutations in *FLNA*,⁵ which suggests that additional loci can be causative for BPNH. Not enough families, however, have been available for linkage studies to identify other loci.

We present the first reported case of a young male who has PMG and BPNH. The patient has seizures and developmental delay. Cytogenetic analysis showed balanced reciprocal translocations in the child and in his father. The translocation breakpoint was mapped and found to interrupt two genes—*mannosidase alpha, class 1A* (*MAN1A2*) and *glutathione S-transferase A2* (*GSTA2*). Deletions of greater than two kilobasepairs (kbp) were excluded, although we could not exclude smaller deletions next to the derivative chromosome 6 breakpoint. On the non-translocated chromosome, no mutations in either of these genes were identified in the *filamin A* related gene, *FLJ13181*, in an adjacent location. In addition, mutations were not identified in any of these genes in six additional unrelated patients with similar brain findings on magnetic resonance imaging scans of the brain; however, these genes remain candidates for this condition and should be screened in a larger patient sample.

Key points

- A translocation breakpoint was mapped in a patient with a brain malformation consisting of bilateral periventricular nodular heterotopia (BPNH) and bilateral perisylvian polymicrogyria (PMG).
- An apparently balanced reciprocal translocation, t(1;6)(p12;p12.2), interrupted two genes, *mannosidase alpha, class 1A* (*MAN1A2*) and *glutathione S-transferase A2* (*GSTA2*). A small deletion on the derivative 6 chromosome breakpoint could not be excluded.
- No potentially causative sequence mutations were found in *MAN1A2* and *GSTA2* in the non-translocated chromosomes of the proband and in six other patients with similar findings on magnetic resonance imaging scans.
- A hypothetical gene, *FLJ13181*, located 245 kilobasepairs upstream of *MAN1A2*, which was homologous to *filamin A*, also showed no causative mutations.
- *MAN1A2* is a particularly well suited potential candidate gene for BPNH and PMG and needs further mutation analysis in a larger patient sample.

MATERIALS AND METHODS

Patient and family history

The proband was born at term after an uneventful pregnancy. He had mildly dysmorphic features including a "box shaped" forehead, pinched nose, protuberant and posteriorly rotated low set ears, micrognathia, and a high arched palate. He presented with a seizure disorder at five months, which included infantile spasms and myoclonic seizures. Neuroradiological findings at age 5.5 months included hypoplasia of the corpus callosum with absence of the

Abbreviations: PMG, polymicrogyria; BPP, bilateral perisylvian PMG; BPNH, bilateral periventricular nodular heterotopia; *MAN1A2*, mannosidase alpha, class 1A; *GSTA2*, glutathione S-transferase A2

rostrum and splenium, bilateral perisylvian polymicrogyria, and bilateral periventricular nodular heterotopia (fig 1). Ophthalmological findings at age seven months included decreased visual behaviour in both eyes and bilateral mild optic nerve pallor.

Six other unrelated patients showed overlap in radiological findings with the proband, including the combination of BPNH and PMG with or without corpus callosum atrophy; these will be presented elsewhere.

Chromosome analysis

We performed cytogenetic analysis on chromosome preparations from peripheral blood samples of the proband and father in two independent clinical laboratories (DNAGene, Houston, TX, USA, and LabCorp, Burlington, NC, USA). We used G-banding for cytogenetic analysis, and the resolution of the studies was 550 bands for both laboratories.

Tissue and DNA samples

We collected blood samples in accordance with University of California's human subjects research policies and obtained approved consent forms (Internal Review Board # S99075+). We isolated DNA from blood and lymphoblast cells with a blood and cell culture DNA Maxi Kit (Qiagen, Valencia, CA, USA). We used buccal swabs to isolate DNA (BuccalAmp DNA Extraction Kit, Epicentre).

Generation of cell hybrids

We used cultured lymphoblastoid cells from the proband to generate cell hybrids from mouse cells (GMP Genetics, Waltham, MA, USA). Purified DNA from 36 cell hybrids and a control mouse cell line were provided for analysis by GMP Genetics.

Identification of cell hybrids containing translocated chromosomes and mapping of translocation breakpoint

We used simple tandem repeat sequences (D1S1596, D1S1627, D1S1653, D1S1677, D1S1679, D1S2134, D1S3462, D1S468, D1S518, D1S551, D6S1006, D6S1017, D6S1040, D6S1051, D6S1056, D6S2436, D6S2439, D6S2522, D6S474, and D6S942 located at 1p32.1, 1p21.1, 1q23.2, 1q23.3, 1q23.3, 1p33, 1q42.2, 1p36.32, 1q31.1, 1p31.1, 6p24.1, 6p21.1, 6q23.1, 6p21.31, 6q16.1, 6q25.2, 6p22.3, 6q27, 6q21, and 6q25.3) to test for heterozygosity in the proband's genomic DNA. Primers were optimised for amplification with polymerase chain reaction (PCR) with a gradient thermal cycler (Eppendorf Mastercycler; Fronine Laboratory Supplies, Riverstone, NSW, Australia) and the Taq PCR kit (Qiagen, Valencia, CA, USA). Reaction mixtures for PCR contained 20 ng DNA template, 1 X buffer, 1 X Q solution, 50 µM deoxynucleotide triphosphate, 0.5 µM primer and 0.5 units Taq. Products from PCR were run on 1–3% agarose gels stained with ethidium bromide. We assessed heterozygosity

Figure 1 Magnetic resonance images of the proband showing brain development defects: axial images that show evidence of BPNH in the form of nodules of tissue isointense with cortical grey matter (white arrows) (A) and of bilateral perisylvian PMG in the form of highly corrugated and infolded grey matter with a widened perisylvian fissure (white arrowheads) (B), coronal image that shows BPNH (white arrows) and PMG (white arrowheads) (C), and sagittal image that shows shortened and thin corpus callosum (arrow) (D).

Primer	Sequence	Tanneal
GSTA2 1F	GTA TTG TTT AAA CTT TGA TTA	53.5
GSTA2 1R	GTG CTA AGG ACA CAT ATT AGT	
GSTA2 2F	TCA TTC ATT CAT TCA TTC AC	53.5
GSTA2 2R	AAC GCA ATC GTA AAT CTG	
GSTA2 3F	GAT AGA GGC CAT TCA ATT	53.5
GSTA2 3R	AGA TAC CCT CAT TAG AGA	
GSTA2 4NF	CAA ATT TAG TCA TTT CAA CAA CCA	61.0
GSTA2 4NR	GTC CCA CCC ACT CAA GGA AGG	
GSTA2 5CF	GAC CAC CGA TGA TCT TC	53.0
GSTA2 5CR	ATA ATT CCA TAT TGG GGT T	
GSTA2 6NF	TTC CTG ATC TGA TTC CTC CCA TTC	64.7
GSTA2 6R	ATG TGG GTC TGC CTC TCT GAA	
GSTA2 7F	CCC TTG ACT TGT TGT TTT AG	53.5
GSTA2 7R	CTT GAC CTC TAT GGC TGG	
MAN1A2 1F	TTC TAA AGT ATT CTC TCC AAG AGC	64.6
MAN1A2 1R	GTA AA	
MAN1A2 2F	ACC GTA CCT TGC TCT GCC AAA T	
MAN1A2 2R	TCC TGG GTT TAA TGG AGA AAC CTT	58.3
MAN1A22R	TCC ATA AAC ACT CCA TAT CAC AAA	
MAN1A2 3F	ATC TGG AAG AAT GGG CAA GGA T	58.3
MAN1A2 3R	TGG AGG TAG AAA ATG GAA ATA G	
MAN1A2 4F	CCC CCA AAG AGA ACA GGA ACC	64.6
MAN1A2 4R	GCA GTT AGA CAT TGT GTC TTT TCA	
MAN1A2 5F	TGG T	
MAN1A2 5R	TTT TCC ACA GGG TGT TTG TGA AA	60.6
MAN1A2 6F	TCC CCT CCA AAA CAA ACA ATG C	
MAN1A2 6R	TGC TCC CGT GTA CCC AGT ACC T	58.3
MAN1A2 7F	TTT TCC CAG AAT AAG AAT ACC	
MAN1A2 7R	CAC AT	
MAN1A2 8F	TGG TCT TTT CCC AAT CCT GTG TC	60.6
MAN1A2 8R	TCT TGT GGC CAA TCT TAA AGG A	
MAN1A2 9F	AAA ACC AAG CCT TGA TGA CAC T	64.6
MAN1A2 9R	CAA CAT CAA GCA AAG AGT TTT	
MAN1A2 10F	CAG A	
MAN1A2 10R	TTG TCT TGT CTT CCC TTG CCT TT	58.3
MAN1A2 11F	ACA CTG TAC ATT CAA TCC ATT AAG	
MAN1A2 11R	CCC AGG TTT TAT AAT GGC CAG A	58.3
MAN1A2 12F	TGC CTA TAT ATT CTC CTT CTT ATT GC	
MAN1A2 12R	TCC TTG CAA ACA AAC ATA AAT GG	60.6
MAN1A2 13F	GGC ATC ATA GGA GGG CAT GAG T	
MAN1A2 13R	TGA AAT TCT ACG GAA TTG CTT ATT	58.3
MAN1A2 14F	TTG T	
MAN1A2 14R	TGA TCA CAC AGC AAA ACC AGT CT	
MAN1A2 15F	TGG TTT TCT GTG CCA TTA AAA GTG	64.6
MAN1A2 15R	AG	
MAN1A2 16F	AAA AGC CGC CCC TTC AAA CTA A	
MAN1A2 16R		

of the proband's genomic DNA at these simple repeat loci with single strand conformation polymorphism polyacrylamide gels that were processed by silver staining. We chose six of the polymorphic markers (D1S1677, D1S3462, D1S551, D1S1006, D6S1051, and D6S1056) to identify the cell hybrids that contained the derivative 1 (der 1) and derivative 6 (der 6) chromosomes with loss of heterozygosity and the presence of concordant amplification of DNA from 6p with 1q and 1p with 6q, respectively.

We designed primers for sequences spanning 1p22.2 to 1q21.1 and 6p21.312 to 6q21.1 with information compiled on the human genome browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>) to map the translocation breakpoint.⁷ DNA extracted from cell hybrids that contained the derivative chromosomes was amplified by PCR, and the presence or absence of a product was detected by agarose gel electrophoresis.

Candidate gene and sequence analysis

We designed allele specific primers to amplify the exons and splice junctions of *GSTA2*. We used PIMER software (version 3.0) to design primers to amplify the exons and splice junctions of *MAN1A2* (table 1) and *FLJ13181* (data not

shown). Products from PCR were digested with exonuclease I (New England Biolabs, Beverly, MA, USA) and shrimp alkaline phosphatase (USB, Cleveland, OH, USA) and purified with QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). We sequenced products with Big Dye Terminator v3.0 (Applied Biosystems, Foster City, CA, USA), purified with Sephadex G-50 (Amersham Biosciences, Piscataway, NJ, USA), and run on a 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence data was processed with Sequencher software (version 1.4.2) (Gene Codes Corporation, Ann Arbor, Michigan, USA). We screened the *FLNA* gene for mutations with denaturing high performance liquid chromatography (Transgenomic, Omaha, NE, USA).

Methylation status

We used CpGenome DNA Modification Kit (Intergen, Burlington, MA, USA) to treat DNA from blood to determine its methylation status.

RESULTS

Elimination of *FLNA* as candidate gene

Screening for *FLNA* mutations in splice junctions and coding exons with denaturing high performance liquid chromatography in the proband showed no mutations causative for BPNH, which suggests that the genes interrupted by the translocation may be related to the proband's phenotype.

Mapping and molecular characterization of the breakpoint

Cytogenetic analysis of the proband and his father showed a 1;6 translocation. One clinical laboratory reported a 46, XY, t(1;6)(p10;q10) translocation, while another reported a 46, XY, t(1;6)(q12;q12) translocation. The cytogenetic result was at the 550 band resolution. Both laboratories indicated that the translocation was balanced cytogenetically at this resolution. The proband's lymphocytes were cultured and used to create mouse cell hybrid cell lines.

The proband's genomic DNA was screened for informative polymorphisms at chromosomes 1 and 6 with 20 simple, tandem, repeat sequences chosen from the Center for Medical Genetics website (<http://research.marshfieldclinic.org/genetics/>). These markers were chosen because they are highly polymorphic, their cytogenetic localisation was a significant distance from the translocation breakpoint, and they were distributed along the length of the chromosomes. Six of the polymorphic markers were chosen to identify cell hybrids that contained the der 1 and der 6 chromosomes, with loss of heterozygosity and presence of concordant amplification of DNA from 6p with 1q and 1p with 6q, respectively, as criteria. Of the 36 cell hybrids, three hybrid cell lines contained der 1 (hybrids 11, 23, and 29), one contained der 6 (hybrid 9), one contained maternal chromosome 1 (hybrid 2), and three contained maternal chromosome 6 (hybrids 12, 19, and 31) (table 2). Two hybrids (13 and 37) showed inconsistent results, which likely resulted from failure of PCR amplification of the maternal sequences at 1p and 6q, respectively.

Translocation breakpoints were determined by molecular analysis and mapping of the breakpoints by PCR analysis. This was done by deriving DNA from cell hybrids and assessing for the positive or negative ability to PCR amplify short fragments in the region harboring the translocation (that is, from 1p22.2 to 1q21.1 and from 6p21.3 to 6q21.1). We used allele specific PCR to amplify sequences because of the presence of highly homologous sequence of five genes and seven pseudogenes near the breakpoint on chromosome 6.⁸ Once the vicinities of the translocation breakpoints were identified, we used PCR amplification across the transloca-

Table 2 Somatic cell hybrid types, presence of maternal (M) chromosomes 1 and 6 and paternal (F) p or q arms on chromosomes 1 and 6 for each hybrid cell line, marker name, and cytogenetic location for each DNA set tested for hybrid cell lines that contained the derivative 1 (hybrids 11, 23 and 29) and derivative 6 (hybrid 9) chromosomes

Hybrid cell line	Marker band and cytogenetic location						Summary
	D1S551 1p31.1	D1S3462 1q23.3	D6S1006 1q42.2	D6S1051 6p24.1	D6S1056 6p21.31	D6S1056 6q16.1	
2	M	M	M				M1
3							
4							
9	F1p					F6q	F1p+F6q
10	F1p			M	M	M+F6q	M6+F1p+F6q
11		F1q	F1q	F6p	F6p		F1q+F6p
12				M		M	M6
13	F1p	F1q	M+F1q	F6p	F6p	F6q	(missing M1p)
14	M+F1p	M+F1q	M+F1q	F6p	F6p	F6q	M1+F1+F6
15	M	M	M	M	M	M+F6q	M1+M6+F6q
16	M	M+F1q	M+F1q	M+F6p	M+F6p	M	M1+M6+F1q+F6p
17		F1q	F1q	M+F6p	M+F6p	M	M6+F1q+F6p
18	M+F1p	M	M	M	M	M+F6q	M1+M6+F1p+F6q
19				M	M	M	M6
21	M+F1p	M+F1q	M+F1q	M+F6p	M+F6p	M+F6q	M1+M6+F1+F6
22	M+F1p	M+F1q	M+F1q	M+F6p	M+F6p	M+F6q	M1+M6+F1+F6
23		F1q	F1q	F6p	F6p		F1q+F6p
24	M+F1p	M+F1q	M+F1q	M+F6p	M+F6p	M+F6q	
25		F1q	F1q	M+F6p	M+F6p	M	M6+F1q+F6p
26	M	M	M	M	M	M	M1+M6
27		F1q	F1q	M+F6p	M+F6p	M	M6+F1q+F6p
28	M	M	M				M1
29		F1q	F1q	F6p	F6p		F1q+F6p
30							
31				M	M	M	M6
33							
34	M+F1p	M	M			F6q	M1+F1p+F6q
35	M+F1p	M	M		M	M+F6q	M1+M6+F1p+F6q
37	M+F1p	M+F1q	M+F1q	M+F6p	M+F6p	F6q	(missing M6q)
38	M+F1p	M+F1q	M+F1q	F6p	F6p	F6q	M1+F1p+F1q+F6p+F6q
39	M	M	M	M	M	M	M1+M6
40	M	M+F1q	M+F1q	M+F6p	M+F6p	M	M1+M6+F1q+F6p
41	F1p	F1q	F1q	F6p	F6p	F6q	F1p+F1q+F6p+F6q
42	F1p	F1q	F1q	F6p	F6p	F6q	F1p+F1q+F6p+F6q
43	F1p	F1q	F1q	F6p	F6p	F6q	F1p+F1q+F6p+F6q
44	M+F1p	M+F1q	M+F1q	F6p	F6p	F6q	M1+F1p+F6q

tion breakpoint on der 1 with a forward primer specific to chromosome 6 sequence and a reverse primer specific to chromosome 1 sequence. The product of PCR produced was sequenced, and the site of transition between chromosome 1 and 6 was detected. We were unable to obtain a PCR product across the translocation breakpoint on der 6, however; possibly because of the repeated sequence in the region. Amplification of chromosome 1 sequence 775 bp from the breakpoint and of chromosome 6 sequence 912 bp from the breakpoint with the der 6 hybrid was successful. A deletion of larger than about 2 kbp was excluded, therefore, but we could not exclude smaller deletions with this method. This 2 kbp interval falls within the introns of the two interrupted genes, so any possible rearrangement is not likely to bear any additional implications beyond what would be expected from interruption by the translocation (see below).

Examination of the genomic sequence in the public database in the regions defined by the breakpoints indicated that the t(1;6)(p12;p12.2) translocation interrupted *mannosidase alpha, class 1A* (*MAN1A2*) at intron 8 on chromosome 1 and *glutathione S-transferase A2* (*GSTA2*) at intron 4 on chromosome 6. We detected products of PCR of exons 9–13 of *MAN1A2* and of exons 5–8 of *GSTA2* in DNA from der 1 hybrids. Similarly, we detected products of PCR of exons 1–8 of *MAN1A2* and exons 1–4 of *GSTA2* in DNA from the der 6 hybrid. As expected, analysis by PCR did not detect exons 9–13 of *MAN1A2* and exons 5–8 of *GSTA2* in the der 6 hybrid or exons 1–8 of *MAN1A2* and exons 1–4 of *GSTA2* in the der 1

hybrids. When we aligned this data with the human genome sequence, the translocation interrupted these genes within intronic sequence and produced a tail to tail orientation of the 3' half of the genes on der 1 and a head to head orientation of the 5' half of these genes on der 6 (fig 2). These translocated genes are unlikely to produce fusion proteins of significance, because the orientations of the genes are inverted with respect to one another.

Mutation analysis of *MAN1A2*, *GSTA2*, and *FLJ13181*

Sequence analysis of products amplified from DNA with PCR from the non-translocated chromosome (with somatic cell hybrids 2 and 12) of *MAN1A2* and *GSTA2* showed no causative mutations in the exons or splice sites. Sequence analysis of the coding exons and splice sites of these genes from six additional unrelated patients with radiological findings similar to those of the proband also showed no deleterious mutations.

We considered the possibility that the translocation could interfere with transcription of neighbouring genes. The genes *CD2*, *IGSF2*, *TTF2*, *GDAP2*, and *WDR3* and the genes that encode the hypothetical proteins FLJ13181, FLJ22418, and FLJ20202 (on the basis of the November 2002 release of the human genome map (<http://www.genome.ucsc.edu>)) were found in the 300 kbp that surrounded the translocation site on chromosome 1. The genes *IL17F*, *MCM3*, *KIAA0057*, *GSTA1*, *GSTA5*, *GSTA3*, *GSTA4*, *ICK*, *FBX09*, *GCM1*, and *HELO1* and the hypothetical proteins C6orf33, FLJ10466, PTD011, and

Figure 2 Schematic diagram that shows the cytogenetic bands and translocation breakpoints on chromosomes 1 and 6 and the resulting derivative 1 chromosome caused by a 1:6 translocation that disrupts *MAN1A2* and *GSTA2*. Exons for *MAN1A2* shown in white and those for *GSTA2* in grey.

FLJ32293 were found on chromosome 6. The gene *FLJ13181* was located 246 kbp upstream of *MAN1A2* and showed protein homology to gene *FLNA*, so it was considered as a candidate gene. Direct sequence analysis of the exons and splice sites of *FLJ13181* in the proband and in all patients with a similar phenotype showed no deleterious mutations.

Assessment of translocation in unaffected family members

Presence of the translocation was assessed in the proband's extended family members with primers that are specific to sequences in intron 4 of *GSTA2* and intron 8 of *MAN1A2* and that detected the translocated chromosome. Amplification of a length specific product with one primer in *GSTA2* and the reverse primer in *MAN1A2* was taken as evidence of the presence of the der 1 chromosome. The der 1 chromosome as assayed by PCR was found in the proband and in his father, paternal grandmother, and half aunt from his paternal grandmother's first marriage. The der 1 chromosome was not found in the proband's mother or his paternal grandfather, half uncle, or half cousin (fig 3).

DISCUSSION

Function of *MAN1A2* and possibility of involvement in proband's phenotype

In the golgi, *MAN1A2* progressively trims α -1,2-mannose residues from Man(9)GlcNAc(2).⁹ Progressive trimming gives Man(5)GlcNAc(2), which in turn initiates branches of complex N-glycans. A multiple adult tissue northern blot showed expression of *MAN1A2* in heart, brain, muscle, pancreas, spleen, thymus, prostate, ovary, intestine, colon, leukocytes, and especially placenta and testis.⁹ The presence of high levels of expression in the placenta is consistent with a crucial role for *MAN1A2* in development.

At least nine congenital disorders of glycosylation are documented in N-glycan assembly and processing, and all are autosomal recessive.¹⁰ Clinical overlap between our

Figure 3 Family tree of proband with family members who tested negative (-) or positive (der 1) for der 1 chromosome with PCR analysis.

patients and these syndromes also exists with several of the reported defects in N-glycan assembly and processing, including epilepsy, mental retardation, optic atrophy, facial dysmorphism, and brain and corpus callosum atrophy.¹⁰⁻¹² This suggests that the underlying disorder in the proband may relate to a glycosylation defect, and experiments are underway to test whether aberrant glycosylation is present in the proband.

Function of *GSTA*

The glutathione S-transferases (GSTs) are enzymes that belong to a supergene family that is expressed in tissue cytosols or membranes; this supergene family can be classified into eight distinct gene families in humans. The main function of GSTs is thought to be the catalysis of the conjugation of electrophilic xenobiotics to glutathione. The *GSTA2* gene is a member of the alpha gene family on chromosome 6, in which five genes and seven pseudogenes reside.⁸ *GSTA2* is a cytosolic isozyme expressed in liver, kidney, lung, small intestine, testis, prostate, pancreas, and trachea, but notably not in brain or fetal brain, as assayed by reverse transcriptase PCR.⁸ Although studies have reported the potential role of genetic polymorphisms in various GSTs in associations with susceptibility to breast cancer, asthma, basal cell carcinoma, and colorectal cancer (reviewed in (11)), no studies have addressed brain developmental issues. Morel and colleagues suggest that the GSTAs arose by gene duplication⁸ and that GSTAs and GSTs have overlapping substrates.¹³ Taken together, *GSTA2* is a less attractive candidate gene for BPNH and PMG than *MAN1A2*.

Absence of detectable mutations in *FLNA* and *FLJ13181* genes in proband

Sheen and colleagues showed that *FLNA* is mutated in at least some men with BPNH.⁵ No mutations in *FLNA* and the hypothetical protein *FLJ13181* that contains *FLNA* homology were identified in the proband on the basis of denaturing high performance liquid chromatography (for *FLNA*) or direct sequence analysis (for *FLJ13181*) of all coding exons and splice sites. Denaturing high performance liquid chromatography, however, will miss some single base mutations, and other types of mutations in the *FLNA* gene that could also underlie the proband's phenotype, such as microduplications, would be undetectable by these methods. In addition, no mutations were identified in *FLJ13181* based on sequence analysis in six additional individuals with a phenotype similar to the proband.

Overview of candidate genes

Several possible explanations might account for the absence of phenotype in other carriers of the translocation. Firstly, we could not exclude a small (<2 kbp) rearrangement of the der 6 chromosome. An undetected rearrangement, deletion, or insertion in the proband that is not present in the other carriers compromises the expression of other genes on der 6. Secondly, female imprinting is a possible explanation. An attempt was made to determine the methylation status of the region 5' of *MANIA2* that contains 145 GPG dinucleotide DNA motifs; however, results were inconclusive. *MANIA2* is not in a known imprinting region on the basis of current data. In addition, the number of transmissions of the translocation (two transmissions from the same woman and one transmission from a man; fig 3) is too small to draw any conclusions about the possible role of female imprinting. Thirdly, multiple enzymatic activities of the various α -1,2-mannosidases imply redundancy in the glycan processing pathway.^{9 14 15} If the proband harbored an additional mutation or mutations in other mannosidase genes inherited maternally, enzymatic redundancies would allow normal glycan processing in the father but would perhaps compromise processing in the proband. The additional possibility of compromised transcription of genes residing within one megabase of the translocation cannot be excluded. This hypothesis postulates that the proband's maternal gene or genes in question would have compromised transcription because of a mutation other than a translocation. Alternatively, the translocation may have no involvement in the proband's phenotype. This possibility is suggested by the lack of a similar phenotype in three other individuals with this same cytogenetic abnormality. Clear examples of translocations that disrupt genes exist, but they are not associated with any phenotype, and this patient may harbor a mutation in a gene as yet unidentified—perhaps in the 1q44qter or Xq28 regions implicated in PMG.^{16 17}

Disruption of the initiation of branching of N-glycans is a serious defect that affects many processes, including brain development. Taken together, the information and data suggest that *MANIA2* remains a viable candidate gene for BPNH and PMG and that verification of mutations in a larger patient set is needed.

NOTE ADDED IN PROOF

The results of studies on transferring glycosylation were unremarkable in the proband, which suggests the absence of major defects of glycosylation.

ACKNOWLEDGEMENTS

NINDS Neuroplasticity of Aging Training Grant (EPL) and NINDS (JGG). We thank Dr Hudson Freeze for helpful comments on the manuscript and the family of the proband for their willingness to participate in this study.

Authors' affiliations

E P Leefflang, S E Marsh, J G Gleeson, Department of Neurosciences, University of California at San Diego, La Jolla, California, USA
E Parrini, F Moro, Neurogenetics Laboratory, Istituto di Neuropsichiatria Infantile (INPE), University of Pisa, IRCCS Fondazione Stella Maris, Pisa, Italy
D Pilz, Institute for Medical Genetics, University Hospital of Wales, Heath Park, Cardiff, UK
W B Dobyns, Departments of Human Genetics, Neurology, and

Pediatrics, University of Chicago, Chicago, USA

R Guerrini, Neurosciences Unit, Great Ormond Street Hospital for Sick Children and Institute of Child Health, University College London, London, United Kingdom

J W Wheless, Department of Neurology and Pediatrics, Texas Comprehensive Epilepsy Program, University of Texas-Houston, Houston, Texas, USA

Correspondence to: Dr Gleeson, Department of Neurosciences, MTF 312, University of California, at San Diego, La Jolla, CA 92093-0624, USA; jogleeson@ucsd.edu

Received: 11 March 2003

Accepted for publication: 19 June 2003

REFERENCES

- 1 Kuzniecky R, Andermann F, Guerrini R. Congenital bilateral perisylvian syndrome: study of 31 patients. The CBPS Multicenter Collaborative Study. *Lancet* 1993;**341**:608–12.
- 2 Kamuro K, Tenokuchi Y. Familial periventricular nodular heterotopia. *Brain Dev* 1993;**15**:237–41.
- 3 Eksioglu YZ, Scheffer IE, Cardenas P, Knoll J, DiMario F, Ramsby G, Berg M, Kamuro K, Berkovic SF, Duyk GM, Parisi J, Huttenlocher PR, Walsh CA. Periventricular heterotopia: an X-linked dominant epilepsy locus causing aberrant cerebral cortical development. *Neuron* 1996;**16**:77–87.
- 4 Fink JM, Dobyns WB, Guerrini R, Hirsch BA. Identification of a duplication of Xq28 associated with bilateral periventricular nodular heterotopia. *Am J Hum Genet* 1997;**61**:379–87.
- 5 Sheen VL, Dixon PH, Fox JW, Hong SE, Kinton L, Sisodiya SM, Duncan JS, Dubau F, Scheffer IE, Schachter SC, Wilner A, Henchy R, Crino P, Kamuro K, DiMario F, Berg M, Kuzniecky R, Cole AJ, Bromfield E, Biber M, Schomer D, Wheless J, Silver K, Mochida GH, Berkovic SF, Andermann F, Andermann E, Dobyns WB, Wood NW, Walsh CA. Mutations in the X-linked filamin 1 gene cause periventricular nodular heterotopia in males as well as in females. *Hum Mol Genet* 2001;**10**:1775–83.
- 6 Fox JW, Lamperti ED, Eksioglu YZ, Hong SE, Feng Y, Graham DA, Scheffer IE, Dobyns WB, Hirsch BA, Radtke RA, Berkovic SF, Huttenlocher PR, Walsh CA. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* 1998;**21**:1315–25.
- 7 Rozen S, Skaletsky HJ. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, eds. *Bioinformatics methods and protocols: methods in molecular biology*. Totowa: Humana Press, 2000:365–86.
- 8 Morel F, Rauch C, Coles B, Le Ferrec E, Guillouzo A. The human glutathione transferase alpha locus: genomic organization of the gene cluster and functional characterization of the genetic polymorphism in the hGSTA1 promoter. *Pharmacogenetics* 2002;**12**:277–86.
- 9 Tremblay LO, Campbell Dyke N, Herscovics A. A. Molecular cloning, chromosomal mapping and tissue-specific expression of a novel human alpha1,2-mannosidase gene involved in N-glycan maturation. *Glycobiology* 1998;**8**:585–95.
- 10 Freeze HH. Update and perspectives on congenital disorders of glycosylation. *Glycobiology* 2001;**11**:129R–43R.
- 11 Cormier-Daire V, Amiel J, Vuillaumier-Barrot S, Tan J, Durand G, Munnich A, Le Merrer M, Seta N. Congenital disorders of glycosylation IIa cause growth retardation, mental retardation, and facial dysmorphism. *J Med Genet* 2000;**37**:866–74.
- 12 Jaeken J, Matthijs G. Congenital disorders of glycosylation. *Annu Rev Genomics Hum Genet* 2001;**2**:129–51.
- 13 Ketterer B. A bird's eye view of the glutathione transferase field. *Chem Biol Interact* 2001;**138**:27–42.
- 14 Lal A, Pang P, Kalelkar S, Romero PA, Herscovics A, Moremen KW. Substrate specificities of recombinant murine Golgi alpha1,2-mannosidases IA and IB and comparison with endoplasmic reticulum and Golgi processing alpha1,2-mannosidases. *Glycobiology* 1998;**8**:981–95.
- 15 Tremblay LO, Herscovics A. Cloning and expression of a specific human alpha 1,2-mannosidase that trims Man9GlcNAc2 to Man8GlcNAc2 isomer B during N-glycan biosynthesis. *Glycobiology* 1999;**9**:1073–8.
- 16 Villard L, Nguyen K, Cardoso C, Martin CL, Weiss AM, Sifry-Platt M, Grix AW, Graham JM Jr, Winter RM, Leventer RJ, Dobyns WB. A locus for bilateral perisylvian polymicrogyria maps to Xq28. *Am J Hum Genet* 2002;**70**:1003–8.
- 17 Zollino M, Colosimo C, Zuffardi O, Rossi E, Tosolini A, Walsh CA, Neri G. Cryptic t(1;12)(q44;p13.3) translocation in a previously described syndrome with polymicrogyria, segregating as an apparently X-linked trait. *Am J Med Genet* 2003;**117A**:65–71.