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

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 reported4; however, most male fetuses with BPNH are not viable. Male patients with BPNH may present with mental retardation, have seizures, 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.5 Many patients with BPNH do not harbor mutations in FLNA,6 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 in a patient with a brain malformation consisting of bilateral periventricular nodular heterotopia (BPNH) and bilateral perisylvian polymicrogyria (PMG).

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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

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.

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).

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) (B).

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, 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 Mastercyler; Fronline 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...
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 (der 1) and 1p with 6q, respectively.

We designed primers for sequences spanning 1p22.2 to 1q21.1 and 6p21.32 to 6q21.1 with information compiled on the human genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway) to map the translocation breakpoint. 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. We identified the polymorphic markers that 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. Once the vicinities of the translocation breakpoints were identified, we used PCR amplification across the transloca-
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, JGSP2, 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, FOX09, GCM1, and HEL01 are the hypothetical proteins C6orf33, FLJ10466, PTD011, and MAN1A2, GSTA2, and FLJ13181.
Bilateral periventricular nodular heterotopia and polymicrogyria with translocation interrupting MAN1A2 and GSTA2 genes

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, and 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). 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. 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. Clinical overlap between our 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. 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. 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. 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 duplication and that GSTAs and GSTs have overlapping substrates. 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. 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 3’ of MAN1A2 that contains 145 GPG dinucleotide DNA motifs; however, results were inconclusive. MAN1A2 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. 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 a region of genes that resides within one megabase of the translocation.

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 MAN1A2 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.

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Authors’ affiliations
E P Leeflang, 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 Pizzi, 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 Whelless, 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; jgleeson@ucsd.edu

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