Am. J. Hum. Genet. 77:120–126, 2005

Report

Homozygous Nonsense Mutations in *KIAA1279* Are Associated with Malformations of the Central and Enteric Nervous Systems

Alice S. Brooks,[1] Aida M. Bertoli-Avella,[1] Grzegorz M. Burzynski,[6] Guido J. Breedveld,[1] Jan Osinga,[6] Ludolf G. Boven,[6] Jane A. Hurst,[7] Grazia M. S. Mancini,[1] Maarten H. Lequin,[2] Rene F. de Coo,[4] Ivana Matera,[6,8] Esther de Graaff,[1] Carel Meijers,[3] Patrick J. Willems,[9] Dick Tibboel,[5] Ben A. Oostra,[1] and Robert M. W. Hofstra[6]

Departments of [1]Clinical Genetics, [2]Radiology, and [3]Pathology and Departments of [4]Child Neurology and [5]Pediatric Surgery, Sophia Children's Hospital, Erasmus MC, Rotterdam; [6]Department of Medical Genetics, University of Groningen, Groningen, The Netherlands; [7]Department of Clinical Genetics, Oxford Radcliffe Hospitals, Oxford, United Kingdom; [8]Laboratorio di Genetica Molecolare, Instituto G. Gaslini, Genova; and [9]Gendia, Antwerp

We identified, by homozygosity mapping, a novel locus on 10q21.3-q22.1 for Goldberg-Shprintzen syndrome (GOSHS) in a consanguineous Moroccan family. Phenotypic features of GOSHS in this inbred family included microcephaly and mental retardation, which are both central nervous system defects, as well as Hirschsprung disease, an enteric nervous system defect. Furthermore, since bilateral generalized polymicogyria was diagnosed in all patients in this family, this feature might also be considered a key feature of the syndrome. We demonstrate that homozygous nonsense mutations in *KIAA1279* at 10q22.1, encoding a protein with two tetratrico peptide repeats, underlie this syndromic form of Hirschsprung disease and generalized polymicrogyria, establishing the importance of *KIAA1279* in both enteric and central nervous system development.

Goldberg-Shprintzen syndrome (GOSHS) (Goldberg and Shprintzen 1981) is a disorder characterized by microcephaly, mental retardation, facial dysmorphism, and Hirschsprung disease (HSCR). GOSHS shares the same accession number (MIM 235730) in the Online Mendelian Inheritance in Man (OMIM) database as the comparable disorder Mowat-Wilson syndrome (MWS) (Mowat et al. 1998). Genetically, however, they are different. MWS occurs as a de novo dominant syndrome, whereas GOSHS is most likely inherited as an autosomal recessive trait, a hypothesis based on the occurrence of the disease in consanguineous pedigrees and in affected siblings with unaffected parents (Hurst et al. 1988; Brooks et al. 1999). MWS is associated with de novo mutations in *ZFHX1B*, located at 2q22 (Amiel et al. 2001; Wakamatsu et al. 2001; Zweier et al. 2002), whereas, for GOSHS, no causative gene has yet been identified. Also, some clinical differences exist. In patients with MWS, neurological abnormalities such as epilepsy (in a high percentage of patients), agenesis of the corpus callosum (in 35% of patients), and cortical malformations (in a minority of patients) have been reported (Amiel et al. 2001; Zweier et al. 2002; Silengo et al. 2004). In the few patients with GOSHS discussed in published reports, neurological symptoms have not been studied in detail, and the cause of the microcephaly and mental retardation remains obscure (Goldberg and Shprintzen 1981; Hurst et al. 1988).

Here, we describe a family with HSCR (an anomaly of the enteric nervous system that is of neural crest origin and is histologically characterized by the absence of ganglion cells in the myenteric and submucosal plexuses) (Okamoto and Ueda 1967) as a variable feature and bilateral generalized polymicrogyria (PMG) (a developmental malformation of the cerebral cortex, characterized by an enlarged number of smaller convolutions or gyri and disruption of the normal six-layered cerebral cortical structure) (Friede 1989) as a constant feature.

Received February 22, 2005; accepted for publication April 19, 2005; electronically published May 9, 2005.

Address for correspondence and reprints: Dr. Robert M. W. Hofstra, Department of Medical Genetics, University of Groningen, Antonius Deusinglaan 4, 9713 AW Groningen, The Netherlands. E-mail: r.m.w.hofstra@medgen.umcg.nl

0002-9297/2005/7701-0012$15.00

The established diagnosis for this family was GOSHS, although bilateral generalized PMG (BGP) has not been reported as part of the syndrome before.

To unravel the genetic basis of GOSHS, we performed a complete genome scan and homozygosity mapping in a large consanguineous Moroccan family reported elsewhere (Brooks et al. 1999). The segregation of the disease in the pedigree is consistent with an autosomal recessive mode of inheritance, with several consanguineous loops (fig. 1), and the family therefore was considered suitable for homozygosity mapping (Lander and Botstein 1987). Informed consent was obtained from the parents of all subjects. In this family, five patients had the cardinal signs characterizing GOSHS (one boy lacked HSCR). One child with long-segment HSCR died in the neonatal period; his DNA was unavailable for our study. When the youngest patient was diagnosed, brain magnetic resonance imaging (MRI) revealed BGP. MRI scans of all living patients also revealed BGP (fig. 2). Gait disturbance, speech defect, excessive drooling, and

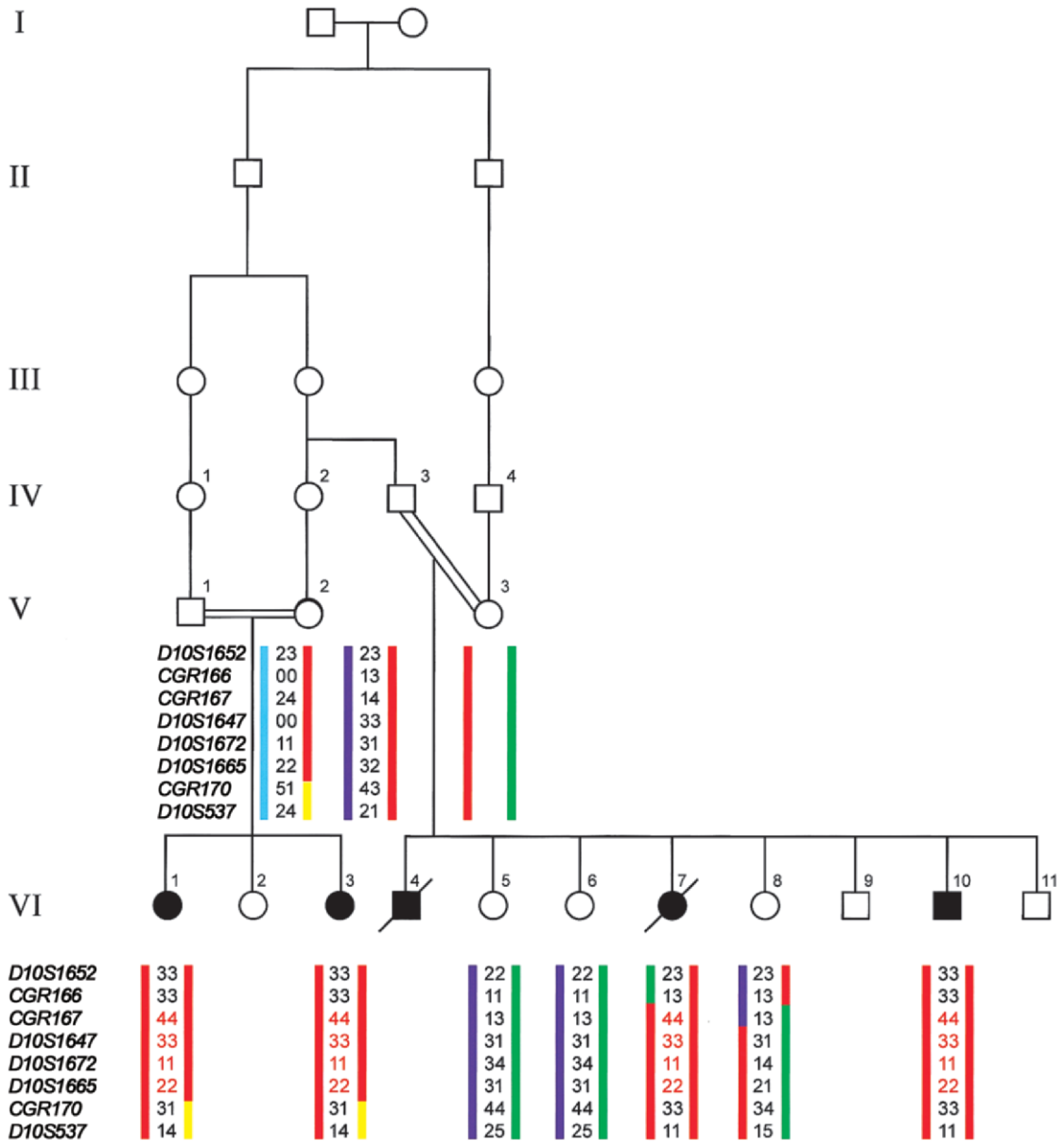

Figure 1 Pedigree structure and haplotypes of the Moroccan family with GOSHS. Patients are represented as blackened symbols. The shared region of homozygosity (*red*) in all affected cases is delimited by markers *CGR166* and *CGR170*. Six of the 11 markers typed for refining the candidate region between marker *D10S1652* and *D10S537* are shown.

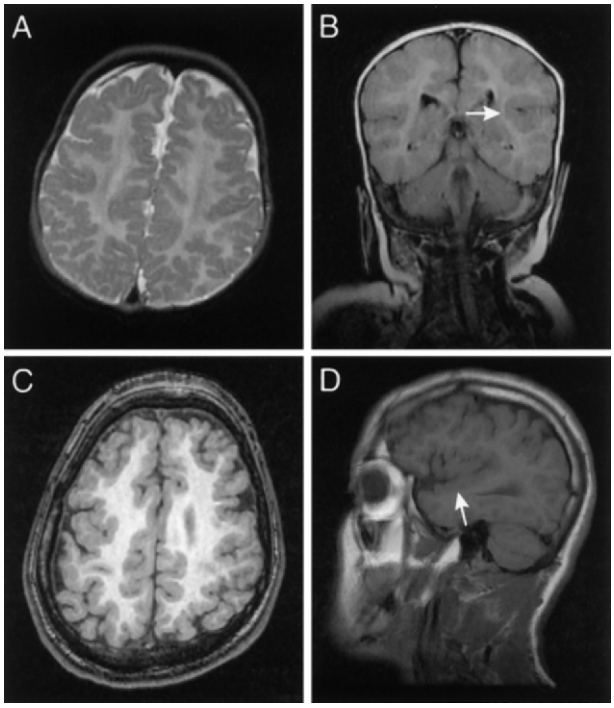

Figure 2 Representative MRIs of patient VI-1 (*panels A* and *B*) and patient VI-10 (*panels C* and *D*), showing BGP. *A,* Axial T2-weighted MRI of patient VI-1 at age 5 mo, showing frontoparietal and occipital bilateral PMG. *B,* Coronal FLAIR image of the same patient showing perisylvian PMG (*arrow*). *C,* T1-weighted axial image of patient VI-10 at age 14 years, showing BGP with distribution similar to that seen in patient VI-1. *D,* Parasagittal T1-weighted image of patient VI-10, showing PMG of the right peri-insular temporal lobe.

pseudobulbar signs indicate diffuse cortical dysfunction including the perisylvian areas.

Genomic DNA was isolated from peripheral leukocytes by use of the method described by Miller et al. (1988). By haplotyping, FISH, and sequence analyses, we excluded the *ZFHX1B* gene as a cause of GOSHS in this family (data not shown). For the systematic genome scan, 381 markers (STRPs) from the ABI Prism Linkage Mapping Set MD-10 (version 2.5), with an average spacing of 10 cM, were tested. DNA amplification was performed using 25 ng genomic DNA in 7.5-μl PCRs containing 1 × PCR Gold Buffer, 2.5 mM $MgCl_2$, 10 μM primer pair mix, and 0.4 U Ampli*Taq* Gold DNA polymerase (Applied Biosystems). Amplification conditions were 10 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 1 min 30 s at 72°C; amplification was ended by a final extension for 5 min at 72°C. PCR products were pooled in panels and were loaded on an ABI 3100 automated sequencer. Data were analyzed using GeneMapper software (version 2.1) (Applied Biosystems).

After this initial screening, a region on 10q21.3-q22.1 was further investigated, since one of the four affected individuals was homozygous by descent for two consecutive markers, *D10S1652* and *D10S537,* whereas the other three patients were homozygous for one of these two markers. No other regions of homozygosity for consecutive markers were identified. We saturated the region between markers *D10S1652* and *D10S537* (12.3 cM) with 11 additional markers; they either were obtained from the Marshfield genetic map (Center for Medical Genetics Web site) or were newly developed. Marker order and genetic distances were obtained from the Marshfield genetic map (Center for Medical Genetics Web site) and the Celera physical map. All patients were homozygous for 7 of these 11 markers. A recombination event between loci *CGR166* and *CGR167* in patient VI-7 defined the centromeric boundary of the genetic interval. The telomeric border was defined by recombination events between markers *D10S1665* and *CGR170* in patients VI-1 and VI-3 (fig. 1). These recombination events reduced the candidate region to a minimum of 2.8 Mb and a maximum of 3.8 Mb.

Parametric two-point and multipoint linkage analyses were performed using the MLINK program from the LINKAGE (version 5.1) software package (Lathrop and Lalouel 1984) and the SimWalk2 program (version 2.9) (Sobel et al. 2002). LOD scores were calculated, under the assumption that the disease phenotype is a rare autosomal recessive disorder with 100% penetrance, with a gene frequency of 1:5,000. No phenocopies were allowed, and equal allele frequencies were used because of the limited number of available independent family members. A significant two-point LOD score was obtained (max LOD score 3.29 and $\theta = 0$ for marker *CGR167*) (table 1), which increased to a maximum mul-

Table 1

Results of Two-Point LOD Score Analysis, Performed for Markers Used in Fine Mapping

	LOD AT θ =						
MARKER[a]	0	.01	.05	.1	.2	.3	.4
D10S196	−13.81	−3.96	−1.52	−.65	−.08	.02	0
D10S1652	−6.96	−2.39	−1.85	−1.36	−.68	−.35	−.15
D10S1743	−4.97	1	1.39	1.31	.9	.46	.14
CGR166	−3.7	.58	1.03	1.02	.73	.4	.13
CGR167	3.29[b]	3.21	2.9	2.51	1.73	1	.4
D10S210	2.59	2.53	2.26	1.92	1.26	.67	.24
D10S1678	1.49	1.45	1.29	1.09	.72	.4	.16
D10S1647	1.84	1.79	1.58	1.33	.86	.47	.19
D10S1672	2.88	2.81	2.52	2.15	1.42	.76	.25
D10S1665	2.77	2.7	2.43	2.08	1.39	.75	.25
CGR172	1.36	1.32	1.17	.97	.62	.32	.11
CGR170	−.28	1.59	1.97	1.88	1.4	.83	.33
D10S676	−.28	1.25	1.64	1.56	1.1	.59	.18
D10S537	−.11	1.45	1.82	1.71	1.2	.65	.21

[a] Marker order follows that of the Celera physical map.
[b] Max LOD score.

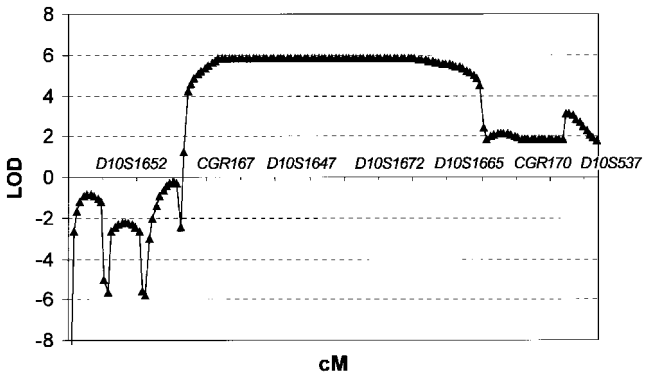

Figure 3 Multipoint LOD score analysis across and beyond the region of homozygosity, flanked by markers *D10S1652* and *CGR170*, with a peak LOD score of 5.9.

tipoint LOD score of 5.9 between markers *CGR167* and *D10S1647* (fig. 3).

The maximum 3.8-Mb linked region of shared homozygosity (defined by *CGR166* and *CGR170*) contained a total of 25 known genes and 5 genes encoding hypothetical proteins, in accordance with the National Center for Biotechnology Information (NCBI) build 34 of the human genome and the Ensembl Genome Browser (table 2). We first sequenced seven functional candidate genes on the basis of their putative involvement in neurogenesis (*CTNNA3, LRRTM3, ATOH7, DDX50, DDX21, NET-7,* and *NEUROG3*), either using cDNA obtained by RT-PCR from a lymphoblastoid cell line of patient VI-10 and from a control or using genomic DNA from both. Mutations were not identified. We then performed a systematic mutational screening of all transcripts from the candidate region. PCR products from either genomic DNA (exons including flanking intronic sequences) or reverse-transcribed products (ORFs) were amplified and purified (ExoSapIT [USB]). PCR primers, which were designed using standard software (Primer3), and conditions are available on request. Bidirectional sequencing was performed using BigDye Terminator chemistry on an ABI 3100 sequencer (Applied Biosystems). With the use of the software package SeqScape (version 2.1) (Applied Biosystems), sequences were aligned and compared with consensus sequences.

Analysis of 98% of all coding sequences from the region revealed one likely disease-causing homozygous nucleotide substitution, 303C→T, in exon 1 of the *KIAA1279* gene (fig. 4*A*). This transversion leads, at the amino acid level, to the replacement of an arginine with a stop codon (R90X), resulting in a shortened protein of 89 aa. The 303C→T mutation showed complete segregation with the disease in the family. One hundred ethnically matched control chromosomes (of North African origin) were screened for the R90X mutation, and none of the controls carried this mutation. Subsequently, one additional family of British Pakistani ancestry, with a phenotype similar to that found in the Moroccan family, was screened for mutations in this gene. This family had four affected individuals with multiple inbreeding loops. Clinical characteristics were consistent with GOSHS, and CT brain scans of this family were described in a previous study (Hurst et al. 1988) and were thought to represent an abnormality of neuronal migration. In the affected individuals, a homozygous G→T transversion was identified at nt 285, resulting, at the amino acid level, in the replacement of a glutamic acid with a stop codon (E84X) (fig. 4*B*). Two different homozygous nonsense mutations in *KIAA1279* in two independent families with GOSHS strongly indicate that *KIAA1279* is the gene responsible for GOSHS.

Table 2

Gene and Marker Positions and Details

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

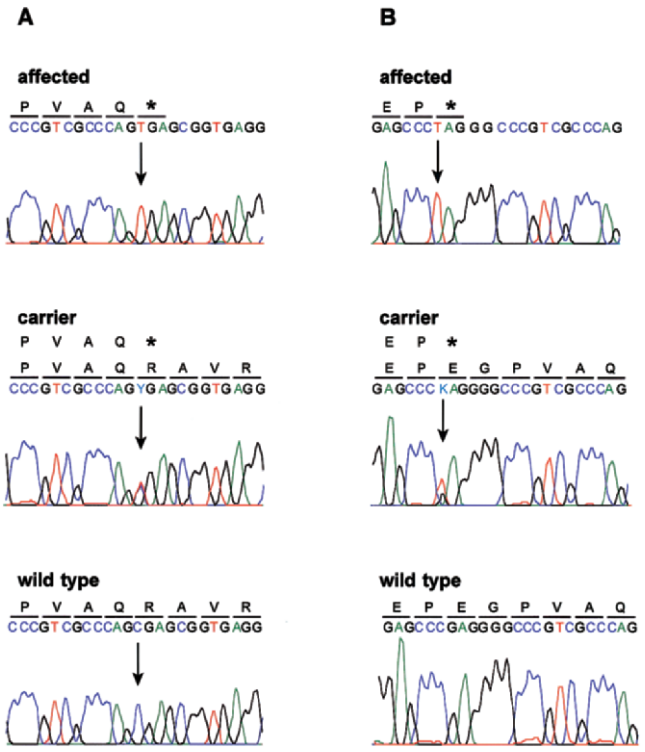

Figure 4 Mutations of *KIAA1279* associated with GOSHS. *A*, Electropherogram of the mutation identified in the Moroccan family. The 303C→T mutation causes the replacement of an arginine with a stop codon (R90X) in exon 1. *B*, Electropherogram of the mutation identified in the Pakistani family. A homozygous G→T transversion at nt 285 results in the replacement of a glutamic acid with a stop codon (E84X) in the patients.

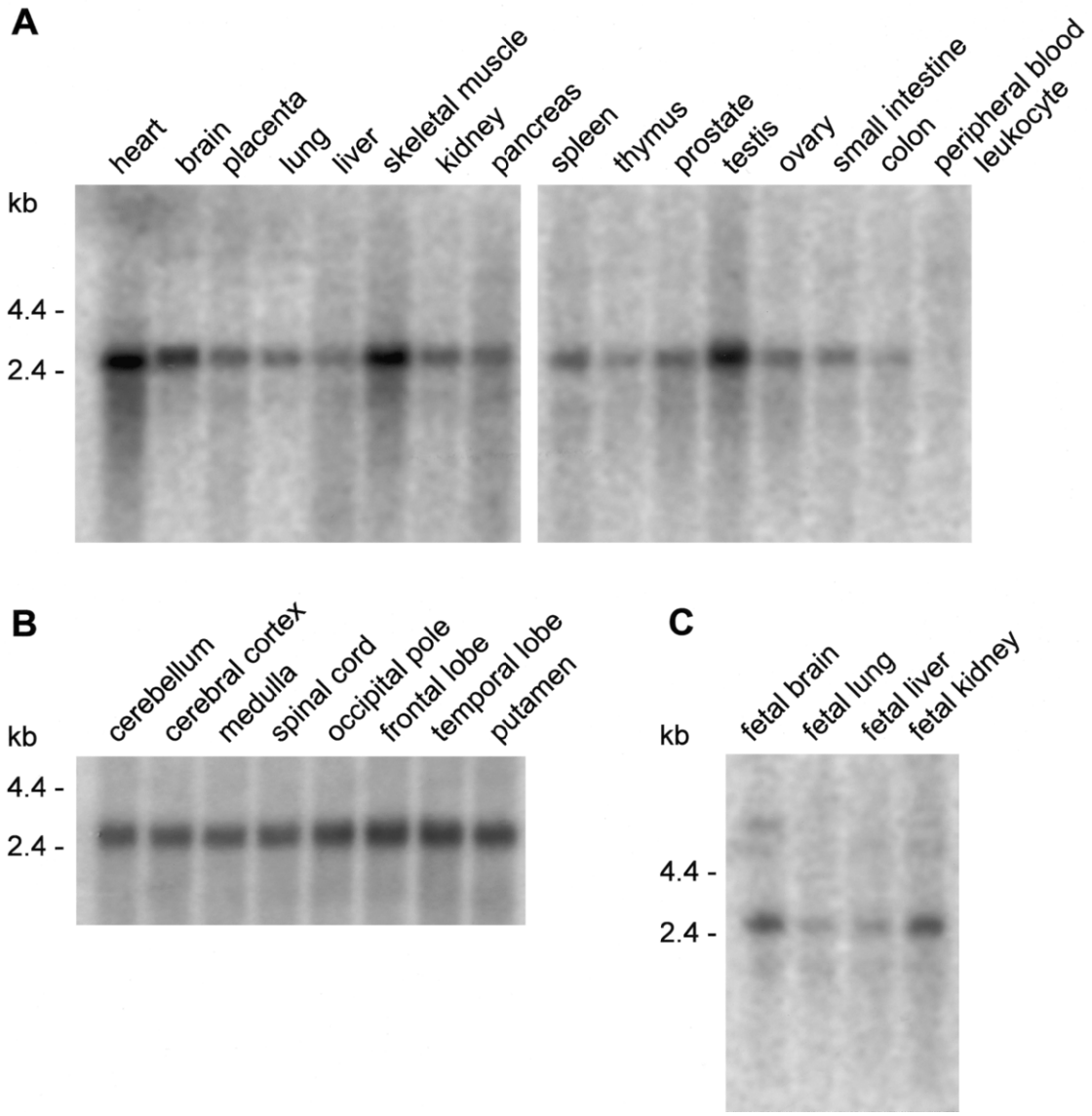

Figure 5 Expression of *KIAA1279* in human adult and fetal tissues. *A,* Multitissue northern blots, incubated with a *KIAA1279* probe, showing ubiquitous expression without a specific pattern (note the lack of expression in blood leukocytes). *B,* Northern blots from adult brain tissues, incubated with a *KIAA1279* probe. *C,* Human fetal multitissue northern blot, showing widespread expression.

KIAA1279 consists of seven exons spanning 28 kb of genomic DNA. The transcript encodes a protein of 621 aa. We identified orthologs of human KIAA1279 in fruit fly, frog, rat, mouse, bee, chicken, and Japanese puffer fish, using BLAST comparison (NCBI Web site). There is strong sequence conservation during evolution; the protein sequence of human KIAA1279 shares 89% amino acid identity with mouse product, 88% with rat product, and 29% with fruit fly product. Multitissue northern blots (both adult and fetal) (fig. 5) showed ubiquitous expression of the 2.4-kb *KIAA1279* mRNA without a specific pattern, with expression lacking only in blood leukocytes (fig. 5*A*). In addition, northern blot hybridization identified *KIAA1279* mRNA molecules in different parts of the adult CNS—namely, cerebellum, cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe, and putamen (fig. 5*B*). This widespread *KIAA1279* expression is consistent with data available in gene expression databases such as the Gene Expression Omnibus, the Human Unidentified Gene-Encoded (HUGE) Protein Database, and the University of California–Santa Cruz (UCSC) Genome Browser. The function of KIAA1279 is unknown. Neither the gene nor its derived protein shows any significant sequence similarity to known human

cDNA or protein sequences. Possible aspects of the molecular function of KIAA1279 may be gleaned from its primary sequence. We predicted protein motifs and domains using the program SMART (Simple Modular Architecture Research Tool). The predicted protein contains two tetratrico peptide repeats (TPRs), which are structural motifs consisting of 34 amino acid residues, in exon 1/2 and exon 4, respectively. Because of these repeats, KIAA1279 may be considered a member of the TPR protein family (D'Andrea and Regan 2003). The most basic function of TPR motifs is to mediate protein-protein interactions. Proteins with TPR motifs are involved in a variety of biological processes, such as cell-cycle regulation, transcriptional control, mitochondrial and peroxisomal protein transport, neurogenesis, and protein folding. Several diseases have been described as being caused by mutations in genes encoding proteins containing TPR domains—for example, Leber congenital amaurosis (*AIPL1*) (Sohocki et al. 2000) and Charcot-Marie-Tooth type 4C neuropathy (*KIAA1985*) (Senderek et al. 2003).

Since mutations in *KIAA1279* are associated with HSCR and PMG, its protein product may play a pivotal role in both peripheral and central nervous system development. PMG is traditionally classified as a result of a disruption such as fetal hypoxic ischemic damage, infection, or exposure to toxic drugs in the second trimester (13–21 wk) (Barth and van der Harten 1985; Barth 2003). However, the identification of *KIAA1279* as the GOSHS gene underlines the importance of genetic factors in the etiology of this neuronal migration disorder of the cortex, as was already proven (Mitchell et al. 2003; Piao et al. 2004) or suggested (Ciardo et al. 2001; Chang et al. 2004) for other forms of PMG. Under the assumption that the main function of KIAA1279 is to bind other proteins, these target peptides might be (known) HSCR- or PMG-associated susceptibility factors. More likely, however, these targets might play a more general role in neuronal development, since a malformation of cortical organization is a constant feature found in the Moroccan family, whereas HSCR is a variable feature. Elucidation of KIAA1279 function and, more important, studies of *KIAA1279* expression and protein interactions may provide new insight into the molecular basis of PMG and HSCR.

Acknowledgments

We thank the patients and their families, for their support and their willingness to donate samples; Jeannette Hoogeboom and Martijn Breuning, for patient care; Jim Barkovich, for help with classification of PMG; Martinus Niermeijer, for his guidance; Elisabeth Lodder and Peter van der Spek, for bioinformatics support; Herma van der Linde and Erik Simons, for technical assistance; and Tom de Vries Lentsch, for help with preparation of the figures. This work was funded, in part, by Nederlandse organisatie voor Wetenschappelijk Onderzoek grants 901-04-210 and 901-04-225 (to R.M.W.H.).

Web Resources

Accession numbers and URLs for data presented herein are as follows:

Celera, http://www.celeradiscoverysystem.com/
Center for Medical Genetics, Marshfield Clinic Research Foundation, http://research.marshfieldclinic.org/genetics/
Ensembl Genome Browser, http://www.ensembl.org/
GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *CGR170* [accession number BV212295], *CGR167* [accession number BV212296], *CGR166* [accession number BV212297], *D10S196* [accession number Z16598], *D10S1652* [accession number Z52339], *D10S1743* [accession number Z53951], *D10S210* [accession number Z16813], *D10S1678* [accession number Z52660], and *D10S1647* [accession number Z52188])
Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/
Human Unidentified Gene–Encoded (HUGE) Protein Database, http://www.kazusa.or.jp/huge/
NCBI, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for GOSHS and MWS)
Primer3, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi
SMART, http://smart.embl-heidelberg.de/
UCSC Genome Browser, http://genome.ucsc.edu/

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