

Mutations in the Gene Encoding Gap Junction Protein α 12 (Connexin 46.6) Cause Pelizaeus-Merzbacher–Like Disease

Birgit Uhlenberg,^{1,3} Markus Schuelke,¹ Franz Rüschemdorf,³ Nico Ruf,³ Angela M. Kaindl,¹ Marco Henneke,⁴ Holger Thiele,³ Gisela Stoltenburg-Didinger,² Fuat Aksu,⁵ Haluk Topaloğlu,⁶ Peter Nürnberg,³ Christoph Hübner,¹ Bernhard Weschke,¹ and Jutta Gärtner⁴

Departments of ¹Neuropediatrics and ²Neuropathology, Charité, University Medical School of Berlin, and ³Gene Mapping Center, Max Delbrueck Center for Molecular Medicine, Berlin; ⁴Clinic of Pediatrics and Pediatric Neurology, University of Göttingen, Göttingen, Germany; ⁵Department of Neuropediatrics, University of Witten/Herdecke, Datteln, Germany; and ⁶Department of Child Neurology, Hacettepe Children's Hospital, Ankara, Turkey

The hypomyelinating leukodystrophies X-linked Pelizaeus-Merzbacher disease (PMD) and Pelizaeus-Merzbacher-like disease (PMLD) are characterized by nystagmus, progressive spasticity, and ataxia. In a consanguineous family with PMLD, we performed a genomewide linkage scan using the GeneChip Mapping EA 10K Array (Affymetrix) and detected a single gene locus on chromosome 1q41-q42. This region harbors the *GJA12* gene, which encodes gap junction protein α 12 (or connexin 46.6). Gap junction proteins assemble into intercellular channels through which signaling ions and small molecules are exchanged. *GJA12* is highly expressed in oligodendrocytes, and, therefore, it serves as an excellent candidate for hypomyelination in PMLD. In three of six families with PMLD, we detected five different *GJA12* mutations, including missense, nonsense, and frameshift mutations. We thereby confirm previous assumptions that PMLD is genetically heterogeneous. Although the murine *Gja12* ortholog is not expressed in sciatic nerve, we did detect *GJA12* transcripts in human sciatic and sural nerve tissue by reverse-transcriptase polymerase chain reaction. These results are in accordance with the electrophysiological finding of reduced motor and sensory nerve conduction velocities in patients with PMLD, which argues for a demyelinating neuropathy. In this study, we demonstrate that *GJA12* plays a key role in central myelination and is involved in peripheral myelination in humans.

Introduction

The hypomyelinating X-linked leukodystrophy Pelizaeus-Merzbacher disease (PMD [MIM #312080]) (fig. 1) is characterized by nystagmus, impaired motor development, ataxia, choreoathetotic movements, dysarthria, and progressive spasticity. The disease is caused by mutations in the *PLP1* gene, which encodes proteolipid protein 1. PLP1 is the major component of myelin in the CNS and is also expressed in myelin of the peripheral nervous system (for review, see Koeppen and Robitaille [2002] and Hudson [2003]). Patients with the PMD phenotype but without mutations or duplications of the *PLP1* gene are considered to have Pelizaeus-Merzbacher-like disease (PMLD [MIM 311601]). No gene locus has been described for this disease entity.

Gap junction proteins are members of a large family of homologous connexins and comprise four transmem-

brane, two extracellular, and three cytoplasmic domains (fig. 2) (Willecke et al. 2002; Nagy and Rash 2003; Nagy et al. 2003). They have been identified in a broad range of mammalian tissues, and most tissues express more than one species of connexin protein. The formation of homo- or heteromeric hemichannels (or connexons) by six connexins permits a high level of diversity in channel composition. Two connexons span the plasma membrane of closely apposed cells and align to intercellular channels, thus allowing for the exchange of small molecules, including second messengers and ions. Gap junctional intercellular communication fulfills multiple functions to meet the specific needs of tissues. Several neurological diseases, such as oculodigital dysplasia (Paznekas et al. 2003) and heterogeneous sensorineural deafness (Rouan et al. 2001), are associated with mutations of genes that encode single members of the gap junction protein family.

For efficient conduction, axons are surrounded by multilamellar myelin membranes that are synthesized by oligodendrocytes and Schwann cells (for review, see Garbern et al. [1997]). Gap junction protein β 1 (GJB1, or connexin 32) is crucial for peripheral myelination, and *GJB1* mutations lead to X-linked demyelinating peripheral neuropathy Charcot-Marie-Tooth type 1

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Address for correspondence and reprints: Dr. Christoph Hübner, Department of Neuropediatrics, Charité, Augustenburger Platz 1, D-13353 Berlin, Germany. E-mail: christoph.huebner@charite.de

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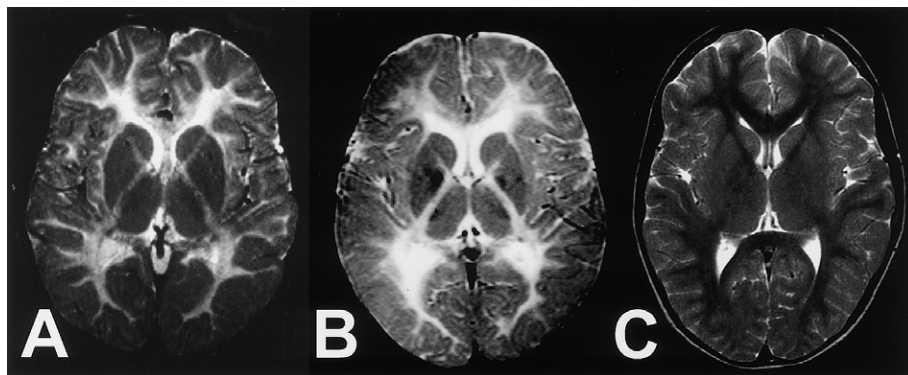


Figure 1 Axial T2-weighted magnetic resonance images of the brain at the level of the basal ganglia. *A*, Patient with PMLD at 6 years of age (patient III:3; fig. 3). *B*, Patient with PMD at 7 years of age. The patients in panels *A* and *B* show nearly identical hypomyelination patterns of central white matter, as indicated by diffusely enhanced signal intensity. *C*, Low signal of normal myelination in an unaffected child.

(CMTX1 [MIM #302800]) (Bergoffen et al. 1993). Similar to *PLP1*, *GJB1* is expressed in both the peripheral and the central nervous system. In consequence, it is not surprising that patients with CMTX1 and specific *GJB1* mutations have both peripheral neuropathy and a mild or transient brain disorder (Paulson et al. 2002; Hanemann et al. 2003; Takashima et al. 2003). Vice versa, a few patients with PMD who have specific *PLP1* mutations have demyelinating peripheral neuropathy in addition to their hypomyelinating leukodystrophy (Garbern et al. 1997). Here, we report that mutations of the gap junction protein $\alpha 12$ gene (*GJA12*, or connexin 46.6) are associated with one type of PMLD and that this type is also accompanied by a mild peripheral neuropathy.

Patients and Methods

Families with PMLD and Collection of DNA

We collected blood samples from patients and family members after obtaining written informed consent, and we isolated DNA from peripheral blood lymphocytes according to standard procedures. The study was approved by the Ethics Review Board at the Charité in Berlin and collaborating institutions, according to the Declaration of Helsinki. We studied a total of nine patients and 20 relatives from six unrelated families. The diagnosis of PMLD was made on the basis of clinical criteria and magnetic-resonance-imaging findings (Cailoux et al. 2000; Schiffmann and Boespflug-Tanguy 2001; Koeppen and Robitaille 2002; Hudson 2003; Plecko et al. 2003). The sensory- and motor-nerve conduction velocities (SCVs and MCVs, respectively) were investigated in patients (table 1) and in heterozygous family members II:2, II:3, and III:2 of family 1 (fig. 3). In all patients with PMLD, PMD was ruled out by mu-

tational analysis of the *PLP1* coding region, the flanking intron sequences, and its promoters. Duplications of *PLP1* were ruled out by Southern blot or real-time PCR. In addition, we found different haplotypes at the *PLP1* gene locus in the two affected brothers (III:1 and III:3; fig. 3) of index family 1 (data not shown). This finding excludes a causative role of *PLP1* in this family.

Genome Scan and Haplotype Analysis

For the whole-genome scan, we used the GeneChip Mapping EA 10K Array (Affymetrix) according to the guidelines of the manufacturer. This early-access version of the Mapping 10K comprised a total of 10,043 SNPs. The mean intermarker distance was 250 kb, equivalent to 0.34 cM. Individuals I:1–I:4, II:1–II:4, and III:1–III:8 of family 1 were genotyped (fig. 3). The following 12 informative SNP markers were used for haplotype reconstruction and analysis: rs958413, rs3902857, rs725033, rs1563353, rs1389742, rs2378627, rs1369847, rs1343743, rs544528, rs559272, rs1321257, and rs1933633 (dbSNP Home Page). PedCheck was used for detection of Mendelian errors (O'Connell and Weeks 1998). Non-Mendelian errors were identified by MERLIN (Abecasis et al. 2002). Parametric linkage and haplotype analysis were performed by a modified version of GENEHUNTER (Strauch et al. 2000), through use of a sliding window with sets of 50 SNPs. All physical positions are derived from National Center for Biotechnology Information build 34, July 2003.

Sequence Analysis

We designed a set of three primer pairs to amplify the single exon of *GJA12* (GenBank accession number NM_020435) and its flanking sequences from genomic DNA (GenBank accession number NT_004559): 1F (5'

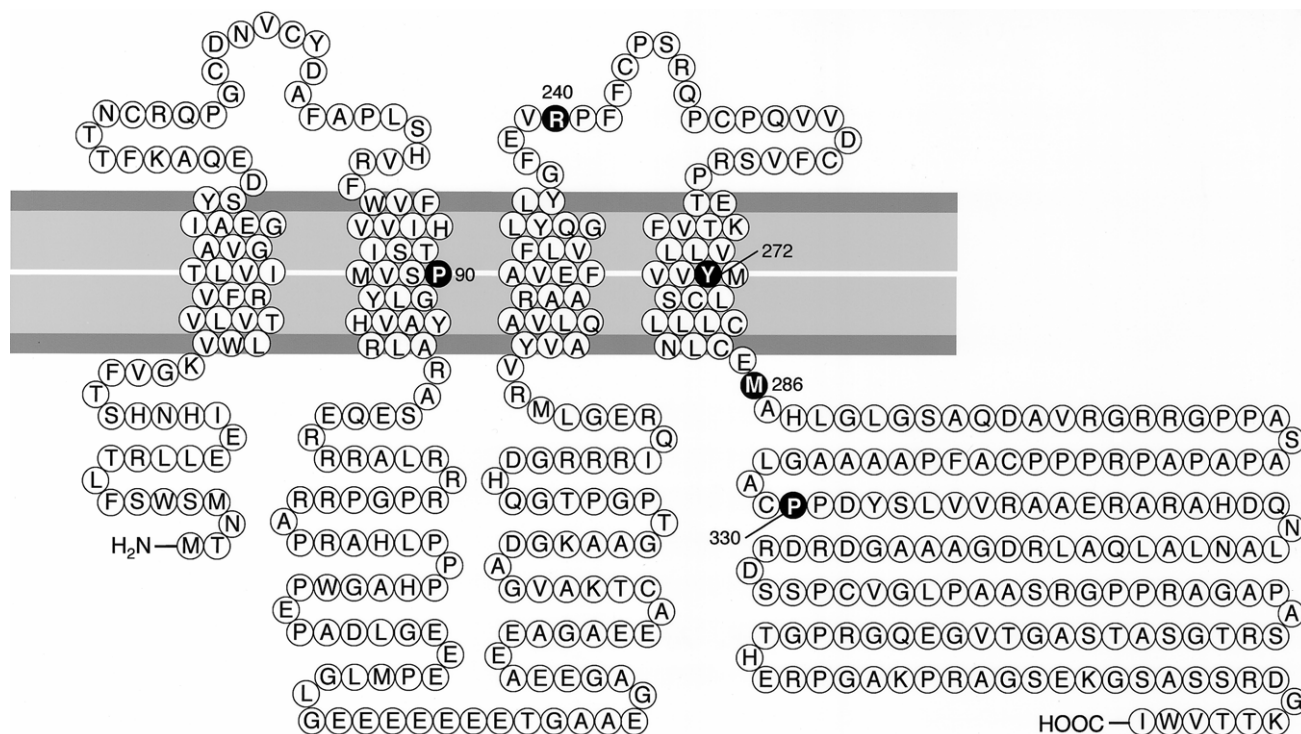


Figure 2 Domains of the *GJA12* gene product. Blackened circles depict the locations of mutations. The missense mutations lie within the second (P90S) and fourth (Y272D) transmembrane and third cytoplasmic (M286T) domains.

TTT AAG GCG GTA AGC TCC AC 3') and 1R (5' CAG CAT GGG CTC CTC CTC 3'), 2F (5' CTG CGA CAA CGT CTG CTA TG 3') and 2R (5' GCC ATC TCA CAG AGG TTG AG 3'), and 3F (5' CCG ACC GGG CAA CAC GAT G 3') and 3R (5' GAG TCT GCC TGA GGC CAC CG 3'). For GC-rich fragments, 1 M betaine was used as an additive. For amplification of the coding region of *GJB1* (GenBank accession number NM_000166) and its flanking sequences from genomic DNA (GenBank accession number NT_011669), we used the following oligonucleotides: 4F (5' TGA CCA TCC TTC CTT TCC TG 3'), 4R (5' ACA TGA AGA CGG CCT CAA AC 3'), 5F (5' GGC TCA CCA GCA ACA CAT AG 3'), and 5R (5' AGT AGC CAG GGA AGG AAG GT 5').

PCR products were directly sequenced using an ABI PRISM 3730 DNA Analyzer and BigDye Terminator Cycle Sequencing Kit version 1.1, according to the protocol of the manufacturer (Applied Biosystems).

We verified the intrafamilial segregation of all mutations by RFLP analysis (fig. 4). We used primer-induced restriction analysis for all mutations without natural restriction sites (the size [in bp] of the fragments yielded after restriction with each endonuclease is given; primer mismatches are underlined): c.268C→T: primers 2F and 1R, *HpyCh4IV*, mutant = 9 + 33 + 38 + 179 bp,

wild type = 9 + 33 + 217 bp; c.718C→T: primers 3F and 3R, *MaeIII*, mutant = 716 + 137 bp, wild type = 853 bp; c.814T→G: primers 3F and 3R, *RsaI*, mutant = 9 + 57 + 58 + 729 bp, wild type = 9 + 57 + 58 + 111 + 618 bp; c.857T→C: primers 6F (5' GTA CCT GCT GTA CGG CTT CG 3') and 6R (5' GCT GCC CAA GCC CAG GTC GGC C 3'), *EagI*, mutant = 166 + 21 bp, wild type = 187 bp; and c.989delC: primers 3F and 7R (5' CGC ACC ACC AGG CTG TAG TCG GCC G 3'), *SfiI*, mutant = 108 + 326 bp, wild type = 25 + 83 + 326 bp. Primer sequences of *TGFB2*, *MARK1*, *CAPN2*, *DEGS*, *ENAH*, and *IMAGE3451454* are available from the authors on request.

RT-PCR

Total RNA was extracted from fresh frozen sural and sciatic nerve specimens from adult healthy individuals by the TRIzol reagent (Invitrogen). RNA from adult brain, spinal cord, and skeletal muscle was purchased from CLONTECH. Integrity of RNA was verified on an agarose gel by visualization of the 5S, 18S, and 28S rRNA bands. Two micrograms of total RNA were subjected to reverse transcription by use of the RevertAid First Strand cDNA Synthesis Kit (Fermentas). Equal loading of template was verified by simultaneous am-

Table 1

Mutations of GJA12 (or CX46.6) and Clinical Data of Patients with PMLD

FAMILY (INDIVIDUAL)	MUTATION (AMINO ACID SUBSTITUTION; CLASS OF MUTATION)	AGE AT ONSET OF NYSTAGMUS (wk)	AGE AT UNASSISTED SITTING ^a (mo)	AGE AT UNASSISTED WALKING ^b (years)	WHEELCHAIR BOUND (years)	TYPE OF SEIZURES (AGE AT ONSET, IN YEARS)	MCV AND SCV, IN M/S (AGE-CORRECTED MINIMAL NORMAL VALUE; AGE) ^c					
							Median Nerve		Tibial Nerve		Peroneal Nerve	
							MCV	SCV	MCV	SCV	MCV	SCV
1 (III:1)	c.857T→C (M286T; missense)	7	15	Not achieved	5.5	Complex partial (8)	59 (51; 21 years)	53 (40; 21 years)	38 (43; 21 years)	34 (41; 21 years)	41 (49; 21 years)	
1 (III:3)		7	6	Not achieved	5.5	Simple partial (12)	39 (51; 14 years)	45 (40; 14 years)	36 (43; 14 years)	ND	37 (48; 14 years)	
1 (III:4)		12	12	5	6.5	Complex partial (7)	67 (51; 18 years)	64 (40; 18 years)	ND	40 (44; 14 years) ^d	48 (49; 18 years)	
2	c.268C→T (P90S; missense)	Congenital	8	Not achieved	6	Complex partial (7)	ND	ND	42 (43; 10 years)	68 (44; 10 years)	ND	
3	c.989delC (frameshift)											
	c.718C→T (R240X; nonsense)	Congenital	Not achieved	Not achieved	1	No seizures	ND	ND	37 (30; 8 mo)	ND	ND	
	c.814T→G (Y272D; missense)											

^a Normal age is 7–8 mo.

^b Normal age is 13–15 mo.

^c ND = not determined.

^d SCV is for the sural nerve.

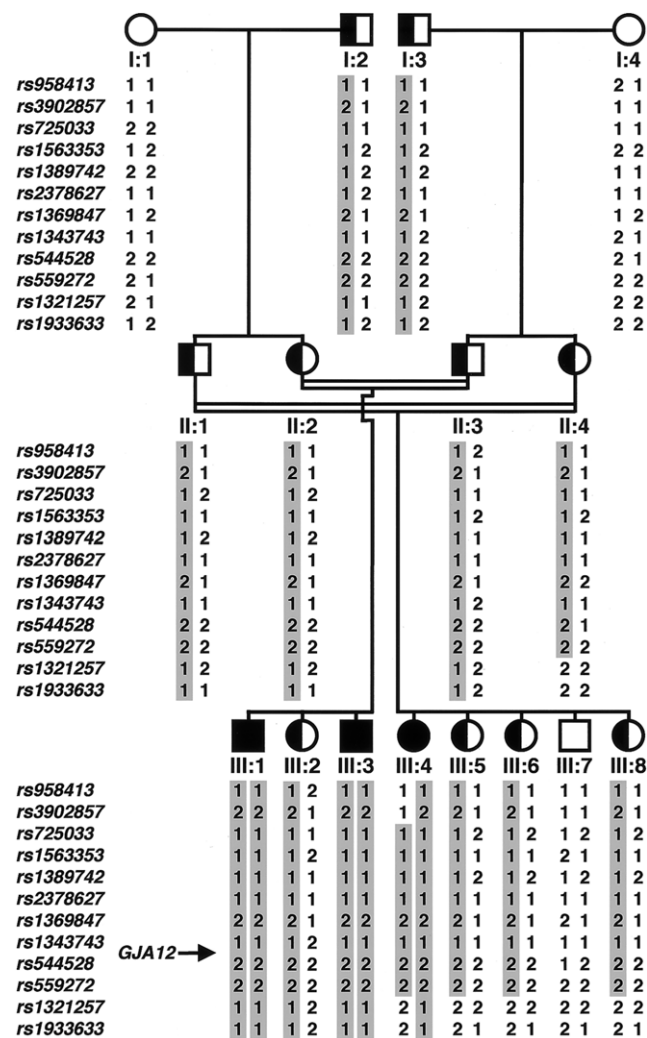


Figure 3 Haplotypes in family 1 with PMLD. Haplotype analysis indicated that the cosegregating segment of the PMLD locus is flanked proximally by marker rs3902857 and distally by marker rs1321257 on chromosome 1q41-q42.

plification of *ACTB* as an internal standard. *GJA12* was amplified by primers 2F and 2R; for *GJB1*, we used primers 8F (5' ACT CCC CCT GCA CAG ACA T 3') and 8R (5' TCT CAT CAC CCC ACA CAC TC 3'). To exclude the provenience of the PCR bands from genomic DNA, we performed control-PCR runs with the above mentioned primers on RT-reaction products in which reverse transcriptase had been omitted. None of the reactions yielded a band. For a relative estimate of the amounts of cDNA, we interrupted amplification of *GJA12* and *GJB1* after 28, 34, and 40 cycles.

Protein Alignment

We performed multiple alignments (fig. 5) (*Gja12*_fugu [GenBank accession number CAAB01002259.1],

*GJA12*_human [GenBank accession number NP_065168], *Gja12*_mouse [UniProt TrEMBL accession number Q9EPM1], and *Gja12*_rat [UniProt TrEMBL accession number Q80XF7]) with the CLUSTAL W program, version 1.74 (Thompson et al. 1994).

Results

Phenotypic Features

All patients with PMLD with *GJA12* mutations (families 1, 2, and 3) showed the characteristic clinical symptoms of the hypomyelinating leukodystrophies PMD and PMLD (fig. 1), such as nystagmus, impaired motor development, ataxia, choreoathetotic movements, dysarthria, and progressive spasticity (Nezu et al. 1996; Lazarini et al. 1997; Cailloux et al. 2000; Schiffmann and Boespflug-Tanguy 2001; Koeppen and Robitaille 2002; Hudson 2003; Plecko et al. 2003). Nystagmus and poor head and trunk control were the presenting symptoms in early infancy (table 1). By the age of 8–15 mo, an impaired motor development became apparent when developmental milestones like unaided sitting and/or walking were delayed or could not be achieved. Only one patient was able to walk a few steps at the age of 5 years. All patients experienced facial weakness. In addition, the patients in families 1 and 2 had focal epileptic seizures prior to adolescence (table 1). In addition to the involvement of the CNS, PMLD associated with *GJA12* mutations seems to be accompanied by a mild peripheral neuropathy. MCVs and SCVs of the lower limb nerves were reduced or slightly below the age-corrected normal values in most patients (table 1). Nerve conduction velocities of the median nerve, available only in members of family 1, were reduced only in patient III:3. Heterozygous individuals had no neurologic symptoms. In contrast to the cranial magnetic resonance imaging (cMRI) of all patients, the cMRIs of two heterozygous healthy members of family 1 (II:2 and II:3; fig. 3) did not reveal any abnormality (data not shown).

The phenotype of the patients with PMLD without *GJA12* mutations was not clearly different from that of patients with *GJA12* mutations. However, two of four patients without *GJA12* mutations did not present with nystagmus in infancy.

PMLD Locus on Chromosome 1q41-q42

Genomewide linkage scanning revealed linkage of PMLD to SNPs on chromosome 1q41-q42. Multipoint linkage analysis yielded a maximum LOD score of 4.83 for SNP markers rs1563353–rs544528. No additional peak with a LOD score value >1 was found in the genome (fig. A1 of the appendix [online only]). Haplotype analysis disclosed recombination events in individual III:4 of family 1 that confined the candidate

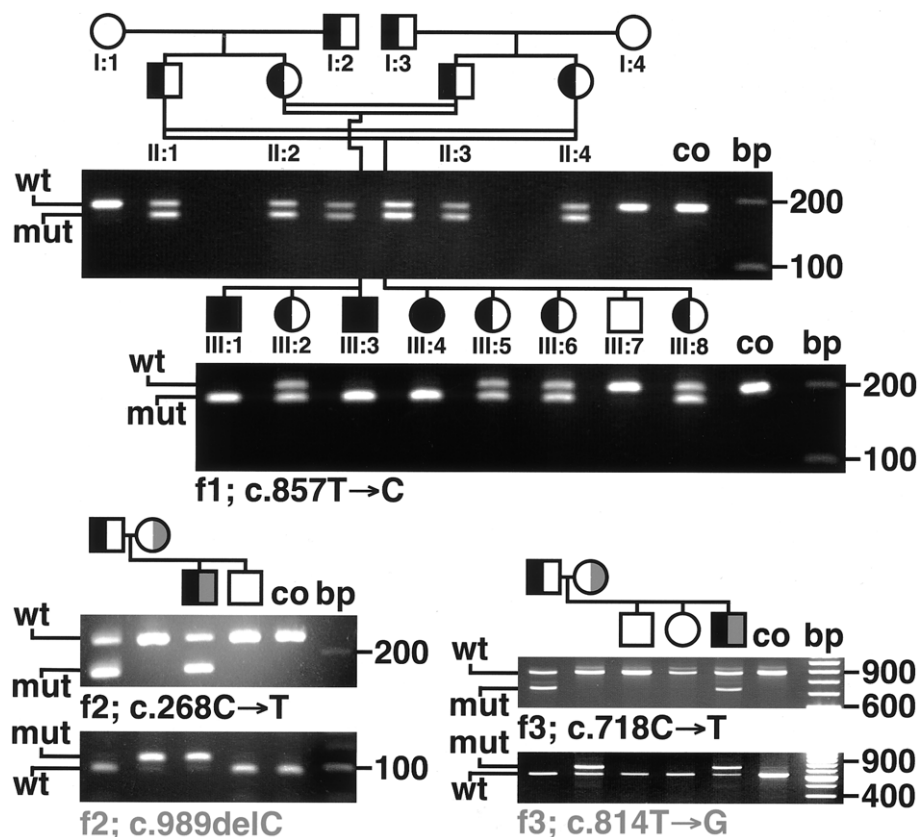


Figure 4 Segregation of *GJA12* mutations (RFLP analysis). In family 2 (f2), the affected boy was compound heterozygous, carrying the paternal c.268C→T missense mutation and the maternal c.989delC frameshift deletion. In family 3 (f3), the affected boy carried the paternal c.718C→T nonsense mutation and the maternal c.814T→G missense mutation. Family numbers correspond to those in table 1. co = control; f1–f3 = families 1–3; mut = mutated *GJA12*; wt = wild-type *GJA12*.

locus to a region distal to SNP rs3902857 (physical map position 215.5 Mb) and proximal to SNP rs1321257 (physical map position 227.3 Mb) (fig. 3). Consistent with parental consanguinity, the affected siblings III:1 and III:3 and the affected cousin III:4 were autozygous for all SNPs within the cosegregating segment. The expression of *Gja12* in murine oligodendrocytes (Menichella et al. 2003; Odermatt et al. 2003) led to the singling out of *GJA12* (physical map position 225.3 Mb) as one of the most promising candidate genes. Mutations in the coding region and its flanking intronic sequences of *TGFB2*, *MARK1*, *CAPN2*, *DEGS*, *ENAH*, and *IMAGE3451454* were ruled out by sequence analysis.

PMLD Gene *GJA12*

GJA12 is composed of a single exon. We analyzed DNA samples from six families with PMLD. In one Turkish consanguineous (family 1; fig. 3) and two German non-consanguineous families, we identified five different *GJA12* mutations (fig. 2 and table 1). We could not find

GJA12 mutations in three other affected families. As expected, patients from the consanguineous family displayed a homozygous mutation, and patients from the nonconsanguineous families were compound heterozygous for the *GJA12* mutations. A homozygous c.857T→C transition predicts a substitution of threonine for methionine in family 1 (M286T). A heterozygous c.268C→T transition (paternal allele) predicts a substitution of serine for proline (P90S), and a heterozygous c.989delC 1-bp deletion (maternal allele) leads to a frameshift and a nonsense peptide of 141 amino acids after amino acid 329 (cysteine) in family 2. A heterozygous c.718C→T transition (paternal allele) represents a nonsense mutation (R240X), and a heterozygous c.814T→G transversion (maternal allele) leads to the replacement of tyrosine by aspartic acid (Y272D) in family 3.

Several findings supported the hypothesis that mutations in *GJA12* are the primary cause for a subgroup of PMLD and are consistent with an autosomal recessive mode of inheritance: (i) no missense mutations were detected in 220 alleles of 110 unaffected individuals, which

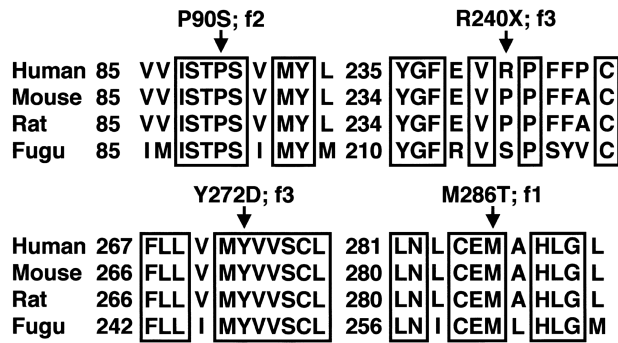


Figure 5 Alignment of selected regions of human *GJA12* with orthologs of other species, including the Japanese pufferfish *Fugu rubripes*. Arrows indicate positions of the missense and nonsense mutations in patients with PMLD. Family numbers correspond to those in table 1. f1–f3 = families 1–3.

rules out common polymorphisms; (ii) *GJA12* mutations segregated with the disease phenotype in all families (fig. 4); and (iii) all residues affected by the three missense mutations are highly conserved (fig. 5).

A silent c.594C→T *GJA12* polymorphism was found in families 3 and 6 and in 3 of 20 healthy individuals. Furthermore, since *Gja12* and *Gjb1* reveal a high level of functional redundancy in murine oligodendrocytes (Menichella et al. 2003; Odermatt et al. 2003), we analyzed not only *GJA12* but also *GJB1*. We did not find mutations in the coding region of *GJB1* or in its flanking intronic sequences in any of the six families with PMLD.

Expression of GJA12 in the Central and Peripheral Nervous System

To further investigate the reduced MCVs in two patients with PMLD (table 1), we examined the *GJA12* expression in comparison with that of *GJB1* by RT-PCR analysis in human sciatic and sural nerve specimens. *GJA12* cDNA could be amplified from sciatic and sural nerves of healthy adults (fig. 6). Intensities of PCR products were compared after various numbers of PCR cycles, to estimate relative amounts of *GJA12* and *GJB1* transcripts. Although *GJA12* and *GJB1* bands both appeared after 28 cycles in brain and spinal cord, they were not detectable in sciatic and sural nerve before 34 cycles or in skeletal muscle before 40 cycles (fig. 6). Thus, the highest *GJA12* and *GJB1* transcript numbers were found in brain and spinal cord, and the lowest were found in skeletal muscle.

Discussion

In this study, we demonstrate that mutations of the *GJA12* gene are associated with one form of autosomal recessive PMLD. In three of six affected families with a

similar phenotype, we could not find *GJA12* mutations, a finding that complies with previous reports that PMLD is a group of genetically heterogeneous diseases (Lazarini et al. 1997; Schiffmann and Boespflug-Tanguy 2001). In addition to the characteristic clinical symptoms, patients with PMLD with *GJA12* mutations had peripheral neuropathy and seizures, both of which occur only sporadically in PMD (Garbern et al. 1997; Cailloux et al. 2000; Koepfen and Robitaille 2002).

Some patients showed reduced nerve conduction velocities, which indicate the presence of a mild peripheral demyelinating motor neuropathy, predominantly of the lower limbs. This complies with our finding that *GJA12* is expressed not only in the CNS but also in sural and sciatic nerve tissue. However, in the murine sciatic nerve, *Gja12* was not found to be expressed, as demonstrated by the absence of fluorescence from the *EGFP* reporter gene, which replaced the *Gja12* coding DNA (Odermatt et al. 2003), as well as by northern blot analysis of the sciatic nerve in wild-type mice (Teubner et al. 2001). Amplification of small amounts of *GJA12* and *GJB1* from human skeletal muscle RNA might be explained by amplification from muscle tissue itself or—more likely—from small amounts of nerve fibers within skeletal muscle. Furthermore, seizures were a frequent finding in our subgroup of patients with PMLD. This observation supports the hypothesis of Samoilova et al. (2003) that gap junctional communication plays an important role in the incidence of seizures. The finding that *GJA12* mutations are associated with one subgroup of PMLD provokes questions concerning those cellular mechanisms that lead to the phenotype of hypomyelinating leukodystrophy. Our data and the studies of other groups suggest mechanisms other than merely a loss of *GJA12* function.

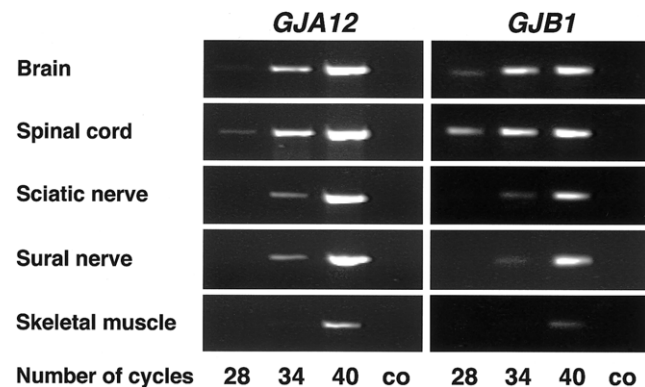


Figure 6 PCR products of *GJA12* and *GJB1* cDNA after various numbers of PCR cycles in human brain, spinal cord, sciatic and sural nerve, and skeletal muscle. *GJA12* and *GJB1* are more highly expressed in brain and spinal cord than in peripheral nerve tissue. Untranscribed RNA was used as a control (co).

Gja12-knockout mice are completely *Gja12*-deficient but clinically normal (Odermatt et al. 2003). In contrast, our patients are affected by missense mutations on one or both alleles and therefore should synthesize mutant GJA12 protein. It has been shown that mutant gap junction proteins may become toxic. For instance, the GJB1 mutant S85C forms functional cell-cell channels, and its hemichannels show an increased opening (Abrams et al. 2002). The GJA12 missense mutation P90S of family 2 (table 1) is located close to the CMTX1-associated S85C missense mutation of the paralogous GJB1. If, in fact, GJA12 mutant proteins became toxic in PMLD, a gain of toxic function might depend on mutant gene dosage, since all heterozygous members of families 1–3 were healthy.

In coexpression studies, it has been shown that mutant gap junction proteins can inhibit functions of other wild-type connexins. Most cell types express more than one gap junction protein species and form gap junctions by single or different protein species (Rouan et al. 2001). Similar to *Gja12* and *Gjb1* in murine oligodendrocytes (Menichella et al. 2003), GJB2 (or connexin 26) and GJA1 (or connexin 43) colocalize in human hyperkeratotic skin (Rouan et al. 2001). In coexpression studies in *Xenopus* oocytes, GJB2 mutants partially blocked the function of wild-type GJA1. Similar negative effects have been shown by *Gja1* mutants on wild-type *Gjb1* (Lagree et al. 2003). The use of site-directed mutagenesis of *GJA12* and *GJB1* for cotransfection in cellular systems will help to clarify the pathogenicity of each connexin mutant.

Both *Gjb1* and *Gja12* are functionally redundant in murine oligodendrocytes (Menichella et al. 2003; Odermatt et al. 2003). Although *Gja12* or *Gjb1* null mutants show no clinical abnormalities, mice lacking both *Gja12* and *Gjb1* develop severe oligodendrocyte death and present with tremor and tonic seizures (Scherer et al. 1998; Menichella et al. 2003; Odermatt et al. 2003). For this reason, we carefully ruled out mutations in the coding region of *GJB1* in all families with PMLD. Under the hypothesis that GJB1 can compensate for the loss of GJA12 not only in mice (Menichella et al. 2003; Odermatt et al. 2003) but also in humans, mechanisms other than loss of GJA12 function should be proposed for our patients with PMLD.

GJB1 mutations lead to demyelinating peripheral neuropathy CMTX1 (Bergoffen et al. 1993; Lin et al. 1999; Hahn et al. 2000). Patients with CMTX1 and specific *GJB1* mutations also present with CNS symptoms (Paulson et al. 2002; Hanemann et al. 2003; Takashima et al. 2003) similar to those seen in PMLD. This is in contrast to patients with deletions of the entire *GJB1* coding sequence who are not affected by brain disorders (Kleopa et al. 2002; Hanemann et al. 2003). These data suggest that other gap junction proteins may

compensate for a loss of central GJB1 function and that distinct GJB1 mutants may have negative effects on oligodendrocytes in humans. We assume that the pathomechanisms in *GJA12*-related PMLD are similar.

GJA12 seems to be more important for oligodendrocyte homeostasis than GJB1. In fact, the central symptoms in CMTX1 (Paulson et al. 2002; Hanemann et al. 2003; Takashima et al. 2003) are transient or milder than in PMLD, and, in contrast to *Gja12*-knockout animals, which show subtle ultrastructural myelin abnormalities in the optic nerve system (Odermatt et al. 2003), *Gjb1*-knockout mice do not display pathological changes of myelination in optic nerves (Scherer et al. 1998).

In summary, mutations of *GJA12* are associated with one form of autosomal recessive PMLD. Since *Gjb1* and *Gja12* are functionally redundant in mice, we favor the hypothesis that the missense mutants found in our patients with PMLD display toxic gain of function in oligodendrocytes, as specific GJB1 mutants may do with regard to central functions of GJA12 in CMTX1.

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Electronic-Database Information

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

dbSNP Home Page, <http://www.ncbi.nlm.nih.gov/SNP/> (for refSNP ID rs958413, rs3902857, rs725033, rs1563353, rs1389742, rs2378627, rs1369847, rs1343743, rs544528, rs559272, rs1321257, and rs1933633)

GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for human *GJA12* mRNA [accession number NM_020435], human *GJA12* genomic sequence [accession number NT_004559], mouse *Gja12* mRNA [accession number NM_080454], *Gja12_fugu* [accession number CAAB01002259.1], human *GJB1* mRNA [accession number NM_000166], human *GJB1* genomic sequence [accession number NT_011669])

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for CMTX1 [MIM #302800], PMD [MIM #312080], PMLD [MIM 311601])

UniProt/TrEMBL, <http://www.ebi.ac.uk/trEMBL/> (for *Gja12_*

mouse [accession number Q9EPM1] and *Gja12_rat* [accession number Q80XF7])

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