Slowed Conduction and Thin Myelination of Peripheral Nerves Associated with Mutant Rho Guanine-Nucleotide Exchange Factor 10

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Slowed nerve-conduction velocities (NCVs) are a biological endophenotype in the majority of the hereditary motor and sensory neuropathies (HMSN). Here, we identified a family with autosomal dominant segregation of slowed NCVs without the clinical phenotype of HMSN. Peripheral-nerve biopsy showed predominantly thinly myelinated axons. We identified a locus at 8p23 and a Thr109Ile mutation in ARHGEF10, encoding a guanine-nucleotide exchange factor (GEF) for the Rho family of GTPase proteins (RhoGTPases). Rho GEFs are implicated in neural morphogenesis and connectivity and regulate the activity of small RhoGTPases by catalyzing the exchange of bound GDP by GTP. Expression analysis of ARHGEF10, by use of its mouse orthologue Gef10, showed that it is highly expressed in the peripheral nervous system. Our data support a role for ARHGEF10 in developmental myelination of peripheral nerves.

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Figure 1  Haplotype analysis of chromosome 8p23 STR markers. a, Pedigree of the family with slowed NCVs. Squares = male; circles = female; unblackened = unaffected; blackened = reduced nerve conduction phenotype; slashed = deceased; diagonal arrow = proband. Blood samples were obtained after informed consent and approval of the Committee for Medical Ethics, University of Antwerp. A genomewide scan was performed using 382 STR markers from the ABI Prism Linkage Mapping, Set MD-10 (Applied Biosystems). Conclusive linkage was found with D8S264. Additional STR markers were used in the fine mapping: three known markers (D8S504, AF009208, and AF009213) and four novel STR markers (designated “STR1,” “STR2,” “STR3,” and “STR4”) (table A [online only]). Genotypes are represented by allele numbers; “0 0” = failed genotype. For each STR marker, a disease haplotype was constructed (box). The presence of the Thr109Ile mutation is indicated by a plus sign (+), the absence by a minus sign (−). The meiotic recombination events, indicated with an arrow, assign the disease locus to the chromosome segment contained between D8S504 and STR2. b, Electron microscopy. Transverse section of superficial fibular nerve shows thinly myelinated fibers (arrows). Scale bar = 10 µm.
we analyzed for mutations by genomic sequencing of exons and flanking splice sites in two affected individuals and two control individuals: KIAA0711 (hypothetical protein KIAA0711 [GenBank accession number NM_014867]), MYOM2 (myomesin [M-protein] 2 [GenBank accession number NM_003970]), CLN8 (ceroid-lipofuscinosis, neuronal 8 [GenBank accession number NM_018941]), DLGAP2 (discs, large [Drosophila] homolog-associated protein 2 [GenBank accession number NM_004745]), and ARHGEF10 (Rho guanine-nucleotide exchange [GEF] factor 10 [GenBank accession number NM_014861]) (intronic primers for ARHGEF10 shown in table B [online only]). A heterozygous mutation, C→T (c.326C→T; g.6110C→T), was observed in exon 3 of ARHGEF10, encoded by 22 exons, as shown by sequence alignment of its 8,467-bp transcript (GenBank accession number NM_014629), with the genomic sequence contained in NT_023744 (fig. 2b). The presence of the mutation was confirmed by Bant digestion of PCR-amplified exon 3, generating one mutant fragment of 329 bp and two wild-type fragments of 172 bp and 157 bp. Complete cosegregation of the mutation was observed in the family (LOD = 9.93) (fig. 1; table 1), and the mutation was absent in 600 control chromosomes. The mutation predicts an amino acid substitution of threonine (Thr) to isoleucine (Ile) at codon 109 (Thr109Ile) of the ARHGEF10 protein (GenBank accession number NP_766339)—showed that Thr109 is a conserved amino acid in all four species (table C [online only]). Screening of patients with different clinical subtypes of Charcot-Marie-Tooth disease did not identify mutations in ARHGEF10. This is not unexpected, given that the individuals in our family were clinically unaffected and had minor disturbances in NCVs and myelination.

We examined expression of ARHGEF10, using its mouse orthologue, Gef10. Alignment of the Gef10 transcript of 4,481 bp (GenBank accession number NM_172751) with the genomic sequence NT_039455 identified 24 exons; exons 1 and 2 were absent from ARHGEF10 (fig. 2b). Multiple-tissue northern blot analysis of Gef10 indicated ubiquitous expression (data not shown). Overlapping primer sets covering the mouse cDNA sequence were used in PCR analysis on cDNA of E13 mouse brain, dorsal root ganglia (DRG), and ventral horn (VH) and demonstrated Gef10 expression in all three neuronal tissues. Extra PCR fragments that indicated the presence of alternative transcripts were observed (fig. 2c–2e). Sequencing of these fragments identified three splice variants of Gef10: one in all three tissues missing exon 4 (fig. 2c), one specific for DRG missing exon 21 (fig. 2d), and one specific to VH, with an insertion of an additional exon of 165 bp between exons 22 and 23 (fig. 2e). Exon 5, which corresponds to exon 3 in ARHGEF10 and contains the Thr109Ile mutation, is present in all three variant transcripts. Whole-mount in situ hybridization experiments in mouse embryos at E8.5 showed Gef10 expression in the neuroepithelium of the meninges, including the optic sulcus (fig. 3a and 3b). At E9.5, high levels of Gef10 expression were detected in the roof plate of the rhombencephalon (fig. 3c and 3d). In E12.5 embryos, Gef10 is ubiquitously expressed, with a pronounced expression in the neuroepithelium of brain vesicles, the neural tube, the ganglia, DRG, and the neural layer of retina (fig. 3e–3b).

ARHGEF10 encodes a guanine-nucleotide exchange factor for the Rho family of GTPase proteins (RhoGEFs) and contains a Dbl homology (DH) domain (codons 177–359), a common feature of all RhoGEFs (Zheng 2001). RhoGEFs activate RhoGTPases by catalyzing the exchange of bound GDP for GTP, which induces a conformational change in the GTP-bound GTPase that allows its interaction with downstream effector proteins (Bourne et al. 1990; Boguski and McCormick 1993; Hart et al. 1994). Within the RhoGEF family, DH domains are invariably followed by a pleckstrin homology (PH) domain, supposed to be involved in subcellular localization of RhoGEFs (Hoffman and Cerione 2002). However, in ARHGEF10, we could not detect a PH-domain consensus motif using several bioinformatic tools (BlastP, ScanProsite, or InterPro). So far, only one
Figure 2  Positional cloning and expression analysis of *ARHGEF10*. a, Linked region on chromosome 8p23. Meiotic recombinants with STR2 and D8S504 defined a critical region of 1.5 Mb. Shaded boxes indicate the five known genes in the region. b, Genomic structure of *human ARHGEF10* and *mouse Gef10*, aligned. The 5′ and 3′ UTRs are light gray; the Dbl domain is dark gray. An arrow indicates the position of the Thr109Ile mutation. c–e, Expression analysis of mouse *Gef10* on cDNA of motor and sensory neurons isolated from VH and DRG of mice embryos, age 13 d. Expression analysis was performed with oligonucleotide primer pairs spread over the proximal, middle, and distal part of the gene. Subsequent direct-DNA sequencing analysis of PCR fragments was performed. M = 100-bp size marker (Invitrogen); Br = mouse brain cDNA (Clontech); VH = VH cDNA; DRG = DRG cDNA; C− = blanco. c, cDNA primer1 and primer2, splicing of exon 4 in the smaller fragment in brain, VH, and DRG. d, cDNA primer3 and primer4, skipping of exon 21 in DRG. e, cDNA primer5 and primer6, additional exon identified, located between exon 22 and 23 in VH. (Primer sequences shown in table D [online only].)

other mammalian RhoGEF family member, p164-RhoGEF, lacking the PH domain has been reported (Rumenapp et al. 2002). ARHGEF10 appears to lack an equivalent protein in *Caenorhabditis elegans*, *Drosophila melanogaster*, *Dictyostelium discoideum*, and *Saccharomyces cerevisiae*, which suggests that the ARHGEF10 signaling pathway is unique to vertebrates. This confirms the overall picture of plasticity, when comparing the RhoGTPases and their interacting proteins between species, with certain species gaining or losing RhoGTPase and RhoGEF family members to give rise to unique sets of signaling proteins (Wherlock and Melior 2002). RhoGTPases play a pivotal role in regulating the actin cytoskeleton, but their ability to influence cell polarity, microtubule dynamics, membrane-transport pathways, and transcription-factor activity is probably just as significant (Etienne-Manneville and Hall 2002).

Recent evidence has implicated RhoGTPases in neuronal morphogenesis, including cell migration, axonal growth and guidance, dendrite elaboration and plasticity, and synapse formation. Several GEFs play a central role in defining the temporal and spatial activation of the corresponding GTPase within neuronal cells (Etienne-Manneville and Hall 2002).

Here, we report a Thr109Ile mutation in *ARHGEF10* cosegregating with slowed NCVs of peripheral nerves
Figure 3  
Expression pattern of Gef10 mRNA in mouse embryo.  

a and b, Frontal view on an E8.5 embryo (a) and blowup of the head region (b) hybridized with the GEF10 probe. GEF10 mRNA is expressed in the neuroepithelium (ne) of brain vesicles and optic stalk (os).  
c and d, Dorsal (c) and lateral (d) views on an E9.5 embryo. High levels of GEF10 mRNA are observed in the thin roof of the rhombencephalon (trr), the neuroepithelium lining the brain ventricles, and, to a lesser extent, in the neural tube (nt).  
e–h, Bright-field (e) and corresponding dark-field image (f) of a transversal section at the level of the epithalamus of E12.5 embryo. Transcripts of GEF10 are present in the ependymal layer (el) of neural tube and brain vesicles, as well as in the trigeminal ganglia (tg) of the head. GEF10 transcripts are also detected in the endolymphatic sac (es) and semicircular canal (ssc).  
g, Dark-field image of a transversal section through the neural tube. Note high expression in the ependymal layer of the neural tube and the DRG.  
h, Transversal section through the region of the eye at E12.5, revealing very high GEF10 expression levels in the neural layer of the retina (nrl). can(XI) = cranial accessory (XI) nerve; tg(V) = trigeminal (V) ganglion; vcg(VIII) = vestibulocochlear (VIII) ganglion; ov = otic vesicle; l = lens; lge = lateral ganglionic eminence; lb = limb bud. The whole-mount and radioactive in situ hybridizations were performed as reported elsewhere (Wilkinson 1992; Dewulf et al. 1995).
without clinical phenotype in a 4-generation family. RhoGEFs have been implicated elsewhere in human genetic disorders. A mutation in the DH domain of FGD1 GEF cosegregates with faciogenital dysplasia, a developmental disorder (Pasteris et al. 1994). Mutations in ARHGEF6 (also known as “αPIX”) are associated with X-linked nonsyndromic mental retardation (Kutsche et al. 2000). Loss-of-function mutations in the ALS2 (alsin) gene cause autosomal recessive amyotrophic lateral sclerosis (ALS2), a neurodegenerative disorder affecting the large motor neurons of the CNS (Hadano et al. 2001; Yang et al. 2001). The identification of ARHGEF10 as a gene implicated in peripheral-nerve conduction raises questions about its role during the development of the peripheral nervous system in vertebrates. All affected individuals in the family had slowed NCVs with normal amplitudes at all ages, which indicates that the phenotype is nonprogressive. Together with the numerous thin myelinated axons in the absence of gross signs of demyelination or axonal degeneration and regeneration in the peripheral nerve biopsy of the proband, these observations are indicative of a congenital nonprogressive phenotype, which suggests that ARHGEF10 is most likely involved in normal development of peripheral nerves, though its actual biological function remains to be elucidated. Also, the upstream signaling cascade necessary for ARHGEF10 activation and the RhoGTPase subsequently activated by ARHGEF10 remain to be clarified.

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