The gene encoding ganglioside-induced differentiation-associated protein 1 is mutated in axonal Charcot-Marie-Tooth type 4A disease

We identified three distinct mutations and six mutant alleles in \(GDAP1\) in three families with axonal Charcot-Marie-Tooth (CMT) neuropathy and vocal cord paresis, which were previously linked to the CMT4A locus on chromosome 8q21.1. These results establish the molecular etiology of CMT4A (MIM 214400) and suggest that it may be associated with both axonal and demyelinating phenotypes.

Charcot-Marie-Tooth disease is the most frequently occurring inherited peripheral neuropathy. CMT is classified in two main groups\(^1\): (i) demyelinating CMT, associated with reduced nerve conduction velocities and segmental demyelination and onion bulb formations, and (ii) axonal CMT, characterized by axonal degeneration without demyelinating lesions and the presence of clusters of regenerative. We diagnosed an axonal CMT phenotype associated with hoarse voice and vocal cord paresis in two small families, LF20 and LF249, and one large inbred family, LF38, with Spanish ancestry. The disease segregates as an autosomal recessive trait. The clinical picture is characterized by onset at childhood with weakness and foot and hand wasting, leading to disability at the end of the first decade. Sensory-nerve action potentials were decreased or absent in all affected individuals. In families LF20 and LF249, we did not register median, ulnar, and peroneal compound motor action potentials (CMAPs). However, we obtained...
CMAPs in axillaris nerves with distal latencies within the normal range (3.7 ms and 3.2 ms, respectively; the normal limit is <5.3 ms). In family LF38, motor-nerve conduction velocities in distal median and ulnar nerves were slightly reduced (41 m s\(^{-1}\), with a CMAP of 1.4 mV, and 42 m s\(^{-1}\), with CMAP of 0.8 mV, respectively) and were normal in proximal median nerves (58 m s\(^{-1}\)). Notably, sural nerve biopsies of two probands showed loss of myelinated fibers and axonal degeneration, with no signs of demyelination and remyelination.

We obtained cumulative lod scores of 6.2 and 4.6 at \(\theta=0.00\) with STR markers D8S286 and D8S164, respectively (Web Table A), originally associated with the CMT4A locus, suggesting that the candidate gene mapped to chromosome 8q21.1 (refs 4–6). We further analyzed the large inbred family for ancestral recombination events by homozygosity mapping (Fig. 1\(^7\)). Loss of homozygosity was observed distal to marker D8S541 in patient VI.1 and proximal to marker D8S551 in patients IV.12 and IV.14, placing the gene within the 2-cM interval flanked by these two markers.

The gene GDAP1 (ref. 8) maps within the genetic candidate region. The homolog Gdap1 is highly expressed in mouse brain and was identified after ganglioside-induced differentiation of the mouse neuroblastoma cell line Neuro2a\(^8\). This gene may be involved in a signal transduction pathway in neuronal development. GDAP1 spans 13.9 kb of genomic DNA, with the coding sequences split into 6 exons. The sequence of GDAP1 contains an ORF of 1,077 nt and encodes a 358-aa protein\(^8\). A 2,505-bp EST (AL110252) located 344 nt downstream of the stop codon of GDAP1, shows two putative polyadenylation signals. We postulated that this sequence may be part of GDAP1 (Fig. 2a). We amplified a 754-bp product from total brain cDNA using nested primers based on the GDAP1 exon 6 and AL110252 sequences (Fig. 2b), suggesting that the EST is an untranslated part of exon 6.

Northern-blot analysis showed the greatest GDAP1 expression in whole brain and spinal cord. We identified a 3.9-kb major transcript, in agreement with the size predicted from the AL110252-containing mRNA (Fig. 2c). We confirmed, by RT–PCR, ubiquitous expression in several human (Fig. 2d) and mouse tissues (Fig. 2e), with apparently predominant expression in nervous system tissues. Amplification of human sural nerve and mouse sciatic nerve transcripts suggests that GDAP1 expression does not occur just in neurons but also in Schwann cells. When expression levels of different nervous tissues are compared, however, GDAP1 expression is higher in central tissues than in peripheral nerves.

We PCR-amplified and directly sequenced the entire coding region of GDAP1, including exon–intron boundaries, in probands of the three families with CMT (Web Table B). We identified (i) six mutant alleles and three distinct mutations; (ii) two nucleotide substitutions; (iii) a C487T transition in exon 4 and a C581G transversion in exon 5 that generate two nonsense mutations, Q163X and S194X, respectively; and (iv) insertion 863insA in exon 6, leading to a frameshift mutation that generates two abnormal amino acids after threonine 288 and terminates the protein at codon 290 (T288fsX290). Family LF38 proband (IV:1) was homoallelic for the Q163X mutation (Fig. 2f). Using HaeIII restriction analysis, we observed complete segregation between the mutation and the disease (Fig. 2g). The proband from family LF249 was a compound heterozygote with respect to Q163X and S194X mutations (Fig. 2f). The index individual from family LF20 was heteroallelic with respect to Q163X and T288fsX290 (Fig. 2f). We confirmed mendelian segregation of the disease in these two families by HaeIII restriction analysis, SSCP analysis and ASO analysis (data not shown). These nucleotide changes were not observed in 134 normal chromosomes.

The amino-acid sequence of GDAP1 shows strong similarity to glutathione S-transferases (GSTs), enzymes that have a role in the detoxification of cells. By secondary structure analysis we detected the \(\beta\)o\(\beta\)o\(\beta\)o\(\delta\) topology of the glutathione (GSH) binding site of the N-terminal thioredoxin-like fold domain of GSTs in
amino-acid residues 26–119, followed by the detection of an α-helical domain II for recognition of xenobiotic substrates. By analyzing the secondary structure, we detected the two domains of GSTs. Amino-acid residues 26–119 show the ββαββα topology of the glutathione (GSH) binding site, and amino-acid residues 210–287 show the α-helical domain II that may recognize xenobiotic substrates. The carboxy-terminal sequence of GDAP1 has two putative transmembrane domains. Phylogenetic analyses showed that GDAP1 belongs to a newly discovered and probably monophyletic group of GSTs that includes the mouse protein and others from Drosophila melanogaster, CG4623, and Arabidopsis thaliana, T14N5.14.

In this issue, Baxter and coworkers have also identified mutations in GDAP1 in the Tunisian families with CMT4A. By analyzing the secondary structure, we detected the two domains of GSTs. Amino-acid residues 26–119 show the ββαββα topology of the glutathione (GSH) binding site, and amino-acid residues 210–287 show the α-helical domain II that may recognize xenobiotic substrates. The carboxy-terminal sequence of GDAP1 has two putative transmembrane domains. Phylogenetic analyses showed that GDAP1 belongs to a newly discovered and probably monophyletic group of GSTs that includes the mouse protein and others from Drosophila melanogaster, CG4623, and Arabidopsis thaliana, T14N5.14.

In this issue, Baxter et al. have also identified mutations in GDAP1 in the Tunisian families with CMT4A described as a severe demyelinating neuropathy of childhood. Genetic data confirms that mutations in GDAP1 may be associated with both axonal and demyelinating phenotypes, as reported for other inherited peripheral neuropathies. We suggest a putative role for GDAP1 in the interaction between Schwann cell and axon that, when interrupted, may cause either axonal degeneration or demyelination in peripheral nerve. Mutated GDAP1 might prevent the correct catalyzing S-conjugation of reduced GSH, resulting in progressive attrition of both axons and Schwann cells.

**Fig. 2** Molecular analysis of GDAP1. a, Diagram showing exon–intron structure. Exons are indicated by black boxes; exon 6 coding sequence is in black and the noncoding sequence is in white. The position of AL110252 EST is indicated as a solid bar. b, Nested PCR between exon 6 and AL110252 (lane 2). c, Northern-blot analysis with a 1,015-bp cDNA probe shows both the major 3.9-kb transcript and a minor 2.9-kb transcript that are more abundant in brain (whole), spinal cord and thyroid gland. β-actin gene ACTB was used as an internal control for the amount of RNA in each sample. d,e, RT-PCR analysis of GDAP1 and GAPDH (control) transcripts in human (d) and mouse (e) tissues. RT(−), no reverse transcriptase was added to the first-strand reaction of the brain RNA. PCR(−), no first-strand template was added to the PCR reaction. f, Direct sequences of GDAP1 mutations identified in the three probands. Mutation changes for exons 4, 5 and 6 are represented on the left side and normal controls are shown on the right. g, Segregation of the Q163X mutation in family LF38 revealed by restriction analysis. PCR amplification product of wildtype DNA produces a 288-bp band. A C487T substitution destroys an HaeIII restriction site that normally generates two fragments of 148 and 140 bp in the wildtype. Affected individuals (filled boxes or circles) show one undigested band of 288 bp, indicating that they are homozygous for the mutation. Obligate carrier parents and carrier sibs show one undigested 288-bp band and the two digested 148- and 140-bp bands. Noncarrier individuals only show the two digested fragments. M, 1-kb plus DNA ladder weight marker; C, undigested control PCR product.

**NCBI reference sequences (RefSeq) for GDAP1:** Genome contig, NT_008209; model nucleotide, Y17849; GDAP1 protein, CA76892.

**GenBank accession numbers.** GDAP1 cDNA, XM_005273; GDAP1 protein, CA76892.

**Note:** Supplementary information is available on the Nature Genetics web site (http://genetics.nature.com/supplementary_info/).

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Small changes in expression affect predisposition to tumorigenesis

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We have used quantitative measures of gene expression to show that constitutional 50% decreases in expression of one adenomatous polyposis coli tumor suppressor gene (APC) allele can lead to the development of familial adenomatous polyposis.

Much of the phenotypic variation among closely related organisms is due to changes in gene expression rather than to alterations in protein sequence. Consequently, it might be expected that variations in disease phenotype would frequently be caused by changes in expression levels rather than structural alterations of genes. However, few examples of genes in which small changes in expression result in severe disease have been documented. Here we show that slightly lower levels of APC expression are associated with a pronounced predisposition to hereditary colorectal tumors.

Notably, in a study designed to detect the causative mutations in individuals with familial adenomatous polyposis (FAP), tentative evidence was obtained for a partial reduction in APC protein expression in one affected person (patient 1). To search for sequence variations in the coding region of APC that might explain this reduction, we isolated the alleles of patient 1 using Conversion technology; however, no sequence variations, other than previously described polymorphisms, were identified in either allele.

To determine whether a reduction in mRNA levels might account for the reduced protein levels, we quantified the relative levels of mRNA transcripts from each APC allele using Digital-SNP. This technique involves dilution of template (genomic DNA in past studies, reverse-transcribed cDNA in this study) so that there is on average less than one template molecule per well in a multi-well plate. PCR products are scored with fluorescent probes that discriminate between the two alleles, and the data is rigorously analyzed using likelihood methods.

Using Digital-SNP, genomic DNA from the proband yielded the expected 50% allelic ratio, but cDNA from lymphoblastoid cells showed a skewed distribution, with a ratio of approximately 66% (Fig. 1). Linkage analyses with this SNP showed that the allele whose mRNA was expressed in lower relative amounts was the one linked to disease (lod score of 3.84 at recombination fraction $\theta$ of zero). Lymphoblastoid-derived RNA from four other affected members of the kindred each had allele ratios of approximately 66% in cDNA, with the linked allele always expressed less, whereas the cDNA from lymphoblastoid cells of 24 unrelated individuals without FAP showed normal allele ratios (Fig. 1).

We found additional evidence for the pathogenic significance of this allele by studying loss of heterozygosity (LOH) of APC in benign tumors from this kindred. DNA from the non-neoplastic fractions of these tumors had balanced (50%) allelic ratios, whereas DNA from the neoplastic fractions of 23 of 28 tumors had allelic loss (Fig. 2). In 22 of the 23 tumors showing LOH, the allele lost in the tumors was the normal allele—that is, the one that was expressed at relatively higher levels and not linked to disease ($P<0.0001$).

To determine whether similar small decreases in expression could be observed in other individuals with FAP, we examined four people with FAP who had no APC abnormalities evident upon IVSP or sequencing of Conversion-separated alleles. One (patient 2) of these four affected individuals had an abnormal, 71% allelic ratio of alleles in cDNA (Fig. 1). No affected relatives were available...