A novel mutation of GDAP1 associated with Charcot-Marie-Tooth disease in three Italian families: evidence for a founder effect

E Di Maria, R Gulli, P Balestra, D Cassandrini, S Pigullo, L Doria-Lamba, M Bado, A Schenone, F Ajmar, P Mandich, E Bellone

SHORT REPORT

C harcot-Marie-Tooth disease (CMT) comprises a group of clinically and genetically heterogeneous disorders of the peripheral nervous system characterised by chronic distal weakness, progressive muscular atrophy, and sensory loss in the distal extremities. On the basis of neuropathological and electrophysiological features, CMT falls into two main subtypes—the demyelinating form, CMT1, and the axonal type, CMT2. CMT1 is characterised by reduced nerve conduction velocities (with values of <38 m/s for the median motor nerve), segmental de- and remyelination, and onion bulb formation. In CMT2, nerve conduction velocities are normal or near normal and nerve pathology shows signs of axonal impairment. The mode of inheritance can be autosomal dominant, X linked, or recessive (autosomal recessive Charcot-Marie-Tooth disease, AR-CMT). For the demyelinating forms of AR-CMT, seven genes have been identified so far.

Methods and results: 76 patients with severe early onset neuropathy and possible autosomal recessive inheritance were screened for mutations. A T>G transversion (c.347 T>G) at codon 116 (M116R) was detected in four affected subjects from three apparently unrelated families. All patients had early onset of disease with pronounced foot deformities and impaired walking. Neurophysiological studies showed an extremely variable expression. Sural nerve biopsies revealed signs of both de-remyelination and axonal impairment, the most prominent feature being a severe loss of larger fibres. Haplotype analysis of the GDAP1 locus demonstrated a common disease haplotype.

Conclusions: The association of the mutation with a common haplotype suggested a common ancestor.

Background: Mutations in a gene encoding a novel protein of unknown function—the ganglioside-induced differentiation-associated protein 1 gene (GDAP1)—are associated with the autosomal recessive Charcot-Marie-Tooth disease type 4A (CMT4A).

Objective: To investigate the role of GDAP1 mutations in causing autosomal recessive neuropathies in an Italian population.

Methods and results: Seventy-six unrelated patients, originating from different Italian regions, were selected on the basis of a severe progressive motor and sensory polyneuropathy with onset in the first decade, absence of clinical and electrophysiological signs in either parent, and exclusion of mutations in the PMP22, MPZ, GJB1, and EGR2 genes.

The entire coding region of GDAP1, including the exon−intron boundaries, was screened for mutation by single strand conformation polymorphism (SSCP) analysis. Direct sequencing of the abnormal polymerase chain reaction (PCR) fragments was carried out on an automated DNA sequencer. Haplotype analysis was done with short tandem repeat (STR) markers D8S551, D8S1829, and D8S441. In addition, a single nucleotide polymorphism (rs3739345), located within intron 1 of the GDAP1 gene, was selected by inspecting the GenBank SNP database. Genotyping of rs3739345 was carried out by direct sequencing of the PCR fragment. The M116R mutation was examined in 157 normal unrelated subjects through primer extension analysis. Detailed protocols are available on request. Neurological, electrophysiological, and neuropathological evaluations were carried out according to standard procedures.

RESULTS

The series of CMT patients was screened for mutations in the entire coding region of GDAP1 by SSCP analysis. The PCR fragment encompassing GDAP1 exon 4 showed two different electrophoretic profiles in both patients and normal controls. Direct sequencing of the exon 4 revealed a G>T transversion at nucleotide 507 (c.507G>T), which did not alter the corresponding serine codon 169, thus revealing the Ser169Ser polymorphism already described. SSCP analysis of exon 3 detected an altered pattern with respect to normal controls in three unrelated index patients. Direct sequencing of the exon 3 revealed a homozygous T>G transversion at position 347 (c.347T>G) predicting an amino acid change from methionine to arginine at codon 116 (M116R). Four individuals belonging to three families carried the homozygous M116R mutation (fig 1). Their unaffected parents were heterozygous carriers. The M116R mutation co-segregated with the disease in all pedigrees and was not revealed in 314 control chromosomes.

As the same GDAP1 mutation was present in three apparently unrelated families and as all patients originated from one Italian region (Campania), we predicted that the mutation might be inherited from a common ancestor. To

Abbreviations: AR-CMT, autosomal recessive Charcot-Marie-Tooth disease; CMT, Charcot-Marie-Tooth disease; GDAP1, ganglioside-induced differentiation-associated protein 1
test this hypothesis, we genotyped available members of the three families for markers D8S551, D8S1829, and D8S541 and for the intragenic marker rs3739345. The M116R mutation occurred on a shared haplotype (boxed in fig 1), which included D8S551, D8S1829, and rs3739345 markers. In family No 2, both affected siblings (patients II:2 and II:3) were heterozygous for the distal marker D8S541. In this family an ancient recombination event may be postulated.

All patients were clinically homogeneous. The onset of the disease occurred in the first decade (range one to seven years) with foot deformities and impaired walking. Wasting of distal muscles in the legs, pes cavus, and marked weakness of the foot dorsiflexors were present. The muscular atrophy showed fast progression, and walking was possible only with external support within the third decade. No affected individuals had hoarseness or vocal cord paralysis.

In the patient from family No 1, gait was acquired at 12 months of age, with bilateral foot drop. The first neurological examination at age 4 showed wasting of distal muscles in the legs, marked weakness of the foot dorsiflexors, bilateral pes varus, and involvement of small muscles of the hands. Deep tendon reflexes were abolished. At age 12, weakness and wasting of the distal leg muscles were more prominent; at age 19 gait was possible only with external supports; and at age 23 he was markedly disabled, walking only with orthoses. Small hand muscle involvement became evident, with atrophy and loss of dexterity.

Both patients from family No 2 showed normal motor milestones. Subject II:2 presented with abnormal gait at seven years of age. Neurological examination at age 14 showed distal muscle wasting in the legs and feet, pes cavus, marked weakness in foot dorsiflexors, gait with bilateral foot drop, and wasting of small hand muscles with normal strength. Deep tendon reflexes were absent. At age 16, he was walking with orthoses and rising from the floor with Gowers' sign. Subject II:3 underwent neurological examination at age 7, showing mild wasting and weakness in distal muscles of the legs and normal strength in the hands. Deep tendon reflexes were normal. At age 9 she had mild pes cavus and gait with foot drop.

After normal early motor milestones, the patient from family No 3 showed abnormal gait at seven years of age. Neurological examination at age 9 showed distal muscle wasting in the legs and feet, pes cavus, marked weakness in foot dorsiflexors, gait with bilateral foot drop, and wasting of small hand muscles with normal strength. Deep tendon reflexes were absent. At age 16, he was walking with orthoses and rising from the floor with Gowers' sign. Subject II:3 underwent neurological examination at age 9, showing mild wasting and weakness in distal muscles of the legs and normal strength in the hands. Deep tendon reflexes were normal. At age 9 she had mild pes cavus and gait with foot drop.

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After normal early motor milestones, the patient from family No 3 showed abnormal gait and frequent falls. On neurological examination at age 9 there was distal muscular atrophy and weakness in the lower limbs, and deep tendon reflexes were absent.
A novel GDAP1 mutation associated with CMT4A

In this report we describe a novel pathogenic mutation (M116R), segregating in three apparently unrelated Italian pedigrees from a restricted geographical area. This mutation is located inside the glutathione S-transferase domain I, which is supposed to be responsible for the functional properties of the protein. Whether GDAP1 is involved in signal transduction or cellular detoxification, as has been suggested, it is likely that the mutated protein is not able to ensure the complex interactions between Schwann cells and neurones, determining the severe, early onset peripheral neuropathy observed in the patients.

The electrophysiological and neuropathological studies disclosed signs of both de-remyelination and axonal impairment, as previously reported in GDAP1 gene mutations. Either in cases with slowed nerve conduction velocities (patients from family 1 and family 3), or in the presence of a normal nerve conduction velocity (patients from family 2), the amplitude of both the motor and sensory action potentials showed a tendency to decrease over time in all the nerves examined, suggesting that the clinical worsening is mainly the result of an ongoing axonal degeneration, as observed in other forms of CMT.

In the present study, 3.9% of subjects (three of 76) carried a novel GDAP1 homozygous mutation. This frequency, however, is biased by the geographical origin of the patients and it probably arises from a founder effect, as suggested by the absence of M116R carriers in a large sample of normal individuals. Further studies on independent sample series are warranted to estimate the disease allele frequency and suggest the criteria for targeted mutation screening of GDAP1. At present, given the lack of specific criteria for CMT4A, GDAP1 might be investigated in peripheral neuropathies with probable autosomal recessive inheritance.

### DISCUSSION

In this report we describe a novel pathogenic mutation (M116R), segregating in three apparently unrelated Italian pedigrees from a restricted geographical area. This mutation was found to be associated with a common haplotype, suggesting a common ancestor.

Consistent with the phenotypes previously associated with GDAP1 mutations, patients with the M116R mutation showed an early onset of the disease and a progression of muscular atrophy leading to loss of independent ambulation within the third decade. Small hand muscle involvement with atrophy and loss of dexterity was evident in all patients in the second decade. None presented with vocal cord dysfunction, which has been described only in patients carrying the Q163X variant. However, genotype–phenotype correlation is hampered by the limited number of GDAP1 mutations so far reported and by uncertainty about the function of the GDAP1 protein. The M116R missense mutation is located inside the glutathione S-transferase domain I, which is supposed to be responsible for the functional properties of the protein. Whether GDAP1 is involved in signal transduction or cellular detoxification, as has been suggested, it is likely that the mutated protein is not able to ensure the complex interactions between Schwann cells and neurones, determining the severe, early onset peripheral neuropathy observed in the patients.

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### Table 1 Electrophysiological features of the patients with the M116R mutation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset (years)</th>
<th>Age at examination (years)</th>
<th>Median nerve</th>
<th>Personal nerve</th>
<th>Median nerve</th>
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<td>CMAP</td>
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<td>CMAP</td>
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<td>50.3</td>
<td>3</td>
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<tr>
<td>Family 3</td>
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CMAP, compound muscle action potential amplitude (mV); MNCV, motor nerve conduction velocity (m/s); ND, not done; NE, not elicitable; SNAP, sensory nerve action potential (μV); SNCV, sensory nerve conduction velocity (m/s).
Competing interests: none declared

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