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GDAP1 mutations in Italian axonal Charcot–Marie–Tooth patients: Phenotypic features and clinical course

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Abstract

Mutations in the ganglioside-induced differentiation associated-protein 1 (*GDAP1*) gene have been associated with both autosomal recessive (AR) and dominant (AD) Charcot–Marie–Tooth (CMT) axonal neuropathy. The relative frequency of heterozygous, dominant mutations in Italian CMT is unknown. We investigated the frequency of dominant mutations in *GDAP1* in a cohort of 109 axonal Italian patients by sequencing genomic DNA and search for copy number variations. We also explored correlations with clinical features. All cases had already been tested for variants in common axonal AD genes. Eight patients (7.3%) harbored five already reported heterozygous mutations in *GDAP1* (p.Arg120Gly, p.Arg120Trp, p.His123Arg, p.Gln218Glu, p.Arg226Ser). Mutations had different penetrances in the families; the onset of symptoms is in the first decade and progression is slower than usually seen in *GDAP1*-related AR-CMT. We show that the relative frequency of mutations in *GDAP* was slightly higher than those observed in *MFN2* and *MPZ* (7.3% vs 6.3% and 5.0%). The relatively milder clinical features and the quite indolent course observed are relevant for prognostic assessment. On the basis of our experience and the data reported here, we suggest GDAP1 as the first gene that should be analysed in Italian patients affected by CMT2.

Keywords: Charcot–Marie–Tooth disease; Axonal CMT; Dominant inheritance; *GDAP1*

1. Introduction

Charcot–Marie–Tooth disease (CMT) is the most common form of inherited peripheral neuropathy. CMT can be classified in two major subtypes: demyelinating (CMT1, CMT4) and axonal CMT (CMT2); an intermediate subgroup also exists. To date, mutations in over 70 genes have been described [1]. Although time is ripe for a targeted molecular approach to the individual forms of CMT through next-generation sequencing (NGS), careful clinical investigations and gene prioritization might still be treasured. In fact the search for a diagnostic algorithm based on ethnic-specific gene mutation frequencies and the knowledge of gene-related clinical features are still needed for cost-efficient mutation detection and for correct interpretation of large-scale NGS data. Mutations in the gene encoding ganglioside-induced differentiation associated-protein 1 (*GDAP1*) are associated with axonal autosomal recessive CMT (AR-CMT) [2,3], demyelinating AR-CMT [4] and intermediate AR-CMT [5,6]; however, they also lead to autosomal dominant CMT (AD-CMT) [7]. AR-CMT is associated with an early and rapidly progressive phenotype, leading to inability to walk by the

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second or third decade. As the majority of *GDAP1* mutations is associated with recessive form, only few data are available about the dominant forms [7–10]. We assessed the relative frequency of AD-CMT in a cohort of Italian CMT2 patients progressively referred to our laboratory. We identified five pathogenic heterozygous *GDAP1* mutations in eight index cases, thus showing that, at least in our cohort, *GDAP1* mutations represent the most common genetic defect in axonal CMT. The counseling and clinical implications of these findings are discussed.

2. Methods

2.1. Participants

From 2000 through 2013, we have collected clinical and neurophysiological data from a large cohort of 1167 Italian CMT cases referred to the Medical Genetics Unit of the University of Genoa. Patients were referred to our specialized center for molecular diagnosis of inherited neuropathies from different Neurological Units scattered all over Italy. Of the whole set of patients, 409 (35%; 225 men, 184 women) were diagnosed as CMT2, according to standard criteria. In particular, a diagnosis of CMT2 was defined when upper-limb nerve conduction velocities (NCVs) were >38 m/s with reduced compound muscle action potential (cMAP). Pure dHMN patients were excluded from the analysis. In our laboratory the standard flowchart for axonal CMT molecular analysis takes into account the following genes in sequence: *MFN2*, *GJB*, *MPZ*, *GDAP1*, *HSPB1*, and *HSPB8*. One-hundred-nine CMT2 index cases (59 men, 50 women; mean age 45 ± 24 years) with autosomal dominant inheritance or isolated cases, in whom *MFN2*, *MPZ*, *HSPB1*, *HSPB8* and *GJB1* mutations were excluded, were recruited for this study.

2.1.1. Standard protocol approvals, registrations, and patient consents

All patients or their legal representatives signed an informed consent form prior to enrollment. This study was in agreement with Region Liguria Ethical Committee statements.

2.1.2. Molecular analysis

Genomic DNA was isolated from peripheral blood samples of patients. PCR (Polymerase Chain Reaction) amplification of the coding exons and exon–intron boundaries of *GDAP1* was performed using primer oligonucleotides previously described [11]. PCR products were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing analysis was performed on an ABI PRISM 3130*xl*-Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Mutations were described according to the HGVS nomenclature (http://www.hgvs.org/mutnomen) and referred on the published mRNA (NM_018972) and protein (NP_061845) sequence of *GDAP1* (www.ncbi.nlm.nih.gov). Variants were confirmed by sequencing a new independent DNA sample, and segregation analysis of the mutations was performed in all available family members. The presence of intragenic deletions of *GDAP1* was investigated by the multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA P353, MRC-Holland, Amsterdam, The Netherlands) in all index cases.

3. Results

3.1. Clinical and genetic findings

We identified five pathogenic heterozygous *GDAP1* mutations (p.Arg120Gly, p.Arg120Trp, p.His123Arg, p.Gln218Glu, and p.Arg226Ser) in 8 families (8 index cases/109, 7.3%) (Fig. 1). All mutations have already been described in association with AD CMT2 [7,8,10–12]; p.Arg120Trp is a mutation with founder effect in the Mediterranean area (Supplementary Data, Table S1). Detailed clinical and electrophysiological studies were performed in a total of 32 subjects from families with *GDAP1* mutations and index cases data are summarized in Tables 1 and 2. Electrophysiological changes are heterogeneous and age-correlated but compatible with axonal neuropathy as the nerves with NCV apparently in the demyelinating range were those in which compound motor action potential was severely reduced (<0.5 mV). In most of the affected individuals the disease onset was in the first decade, as already described [12], and walking difficulties were the most common clinical manifestations at onset. Disease progression was very slow with all patients remaining ambulatory at the time of this report with an ambulation index score in the range between 0 and 3. The overall severity was milder than what is usually seen in patients harboring AR-*GDAP1* mutations as weakness and atrophy was mainly restricted to distal muscles in the limbs. There was a considerable inter- and intrafamilial phenotypic variability among mutation carrier individuals even though many carried the same mutation. Family CMT-5, carrying the p.Arg120Gly substitution, has been previously reported elsewhere [11]. Two other families are peculiar and are described in detail below. Family CMT-4 originates from Southern Italy. All four affected subjects carry the mutation p.Arg226Ser, previously reported by Crimella and colleagues [10] (Fig. 1C). The 46-year-old index case is a woman who complained of distal paresthesia and dysesthesia since she was 35 years old. Neurological examination disclosed Adie's pupil, absence of deep tendon reflexes at ankles and reduced sensation to pain in the feet. The same clinical picture was present in her 31-year-old daughter. Two younger sons (aged 29 and 16, respectively) were asymptomatic and had a normal neurological examination, but NCVs were consistent with a length-dependent axonal sensory neuropathy. The index case and her daughter underwent quantitative sensory testing (QST), thermoregulatory sweat test (TST), dynamic sweat test (DST) and skin biopsy. They showed distal anhidrosis by TST and DST supported a postganglionic sudomotor dysfunction, more evident in the lower limbs. Ultrastructural examination of a skin biopsy from fingertip, thigh and leg was processed using indirect fluorescence technique to mark sensory and autonomic nerve fibers. We found a moderate loss of epidermal nerve fibers (ENFs) in the fingertip and thigh of the proband and a loss of ENF limited to the fingertip of the daughter. In both we found a moderate loss of autonomic nerves to dermal annexes (erector pilorum muscle, sweat glands, vessels) involving mostly sudomotor nerves (Fig. 2). Family CMT-8 comes from Sicily and includes three symptomatic carriers