A Novel Mutation of GDAP1 Associated with Charcot-Marie-Tooth Disease in An Iranian Family

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Abstract

As a result of higher distributed consanguinity in the Mediterranean region and the Middle East, autosomal-recessive forms of Charcot-Marie-Tooth (ARCMT) are more common in these areas. CMT disease caused by mutations in the ganglioside-induced differentiation-associated protein 1 (GDAP1) gene is a severe autosomal recessive neuropathy resulting in either demyelinating CMT4A neuropathy or axonal neuropathy with vocal cord paresis. The patient was an 8-year-old boy with AR inheritance that showed some delayed achievement of motor milestones, including walking, also bilateral foot drop, wasting of distal muscles in the legs, pes cavus and marked weakness of the foot dorsiflexors. He had no hoarseness or vocal cord paralysis. Total genomic DNA was extracted from whole peripheral blood of the patient and his family by using standard procedures. PCR- sequencing method were used to analysis the whole coding regions of the GDAP1 gene. A novel homozygote insertion of T nucleotide in codon 34 was detected (c.100_101insT) that probably led to an early stop codon. This mutation may be associated with a common haplotype, suggesting a common ancestor that needs further investigation in the Iranian population.

Keywords: ARCMT; CMT 4A; GDAP1; Novel mutation

Introduction

Charcot-Marie-Tooth (CMT) is one of the sensory and motor neuropathies and the most common type of hereditary neuropathy (1). CMT disease is a genetically and clinically heterogeneous disease in which genotype-phenotype correlations are difficult to diagnosis. Both phenotypic features and disease severity can either be consistent or vary widely both within and among families. Typical symptoms include distal muscle weakness and loss of muscle bulk which are seen together with mild to moderate sensory loss, decreased or no deep tendon reflexes, high-arched feet and skeletal deformities such as pes cavus (2). The CMT hereditary neuropathies are classified based on the inheritance mode, causative gene or chromosomal location. CMT is classified into two CMT1 (demyelinating form) as autosomal-dominant inherited with severely reduced nerve conduction velocities (NCVs) and CMT2 (axonal form) (3). The CMTX form is when patients have an X-linked inheritance pattern and CMT4 in case the pattern of inheritance is autosomal recessive (4). A number of demyelinating autosomal-recessive CMT (ARCMT) disorder have been associated to some genes including: GDAP1, EGR2, KIAA1985, MTMR2, MTMR13, NDRG1,

PRX, FIG4, GD4, and CTDP. In communities with a high percentage of consanguineous marriages, ARCMT is likely to account for 30-50% of all CMT cases (5). We should point to the fact that patients in the Mediterranean region and the Middle East with autosomal-recessive disease are usually noticed as isolated cases due to the insignificant size of the sibships. ARCMT forms are usually characterized by an earlier onset and a more severe disease course and may be demyelinating, axonal or even intermediate as in dominant forms. Predominantly in inbred families generating from the Middle East several genes have been identified. All mutations (nonsense, frameshift and deletions) in CMT disorder certainly cause loss of gene function. Primary peripheral demyelinating neuropathy is characterized by demyelination with severely reduced motor nerve conduction velocity (MNCV) cause to a partial or complete loss of the myelin sheath. In CMT4 and CMT1 a slow nerve conduction, which is lower than the 38 m/sec normal minimal threshold–usually 10 to 30 meters per second– is seen. Nerve biopsy is a great diagnostic value in case of a demyelinating process (CMT4 subtypes). CMT4 with GDAP1 mutation is associated with a severe phenotype described by a rapidly progressive weakness due to inability to walk (6) that begins at the age of less than 3 years and is frequently due to recessive demyelinating intermediate and/ or axonal forms of CMT (7-9) with a median MNCV of 27-35 m/s. CMT4A neuropathy or axonal neuropathy with vocal cord paresis which maps to the CMT4A locus on chromosome 8q21.1 and has six exons with 4.1 kb transcript encoding an ORF of 358 amino acids (7, 8, 10).

Case presentation

The patient was an 8-year-old Iranian boy that had a pedigree compatible with AR inheritance (Fig 1) (the patient was symptomatic at the first age). He showed some delayed achievement of motor milestones, including walking (independent walking was achieved at 20 months of age). The onset of the disease occurred in late infancy (around 18th month ages) with impaired walking. Bilateral foot drop, wasting of distal muscles in the legs, pes cavus and marked weakness of the feet dorsiflexors developed gradually. The muscular atrophy showed fast progression and walking was possible only with external support. He had no hoarseness or vocal cord paralysis. Upper extremity symptoms began later with muscle wasting and finger contractions (mild claw hand deformity). As the disease progressed, the distal upper extremities also became severely affected and proximal muscles became weak too. The patient had been wheelchair bound at the age of 8 years old. Speech, hearing, vision and intelligence were intact. Deep tendon reflexes were abolished. The latest electro-diagnostic study performed at 8 years of age showed marked slowing of nerve conduction velocity (NCV) (median NCV was 25 m/s) and sensory NCV – H and F waves – was not obtained. The CMAP amplitude was 1MV. The electrophysiological study was compatible to mainly demyelinating polyneuropathy.

Electrodiagnostic Testing

Regarding the pattern of inheritance and the electrodiagnostic finding (mixed type demyelinating and axonal) a CMT type 4A was suspected in this child so he was investigated for mutation in GDAP1 gene (the commonest autosomal recessive type of CMT4).

DNA Extraction

Peripheral whole blood samples were extracted from the patient and his family by salting out method to isolate total genomic DNA (11).

Mutation Analysis of the PMP22 and Cx32 gene

The patient was tested for PMP22 duplication (PMP22) and connexin 32 gene (Cx32), but both tests were negative.

Sequencing of the GDAP1 gene

PCR amplification and direct sequencing methods were used for the detection of mutations in whole coding regions of the GDAP1 gene. Overall, a novel insertion in the first exon of the GDAP1 gene in one of the Iranian families (c: 34 insT) were detected in a proband patient (Fig2).

Discussion

These disorders show a wide phenotypic and genetic heterogeneity. A novel mutation (c.100_101insT) in the GDAP1 gene in an Iranian patient was identified. The patient showed normal motor milestones before the first year of age followed by early development of

severe foot deformity at the age of 4 years.

This mutation showed an early onset of the disease and a progression of muscular atrophy leading to loss of ambulation within the first decade. Involvement of the upper extremities (small hand muscles) was evident at the end of the first decade. c.100_101insT mutation was not associated with vocal cord involvement as Birouk et al. has reported in Q163X variant (12).

Mutation in GDAP1 gene may weaken the correct catalyzing S conjugation of reduced glutathione leading to progressive attrition of the axon and/or schwann cell and mutations in this gene causes demyelinating, intermediate or axonal forms of peripheral neuropathy in CMT disorder without severe CNS phenotype (13). GDAP1 mutations with autosomal recessive inheritance are associated with a rapidly progressing weakness that is occurred before the age of 3 years and the patient is not able to walk by the second or third decade of life (6). The demyelinating form of CMT disorder leads to reduced motor and sensory NCV and weakness and atrophy of the foot and hands, but in the patient with axonal CMT phenotype it is characterized by vocal cord paresis, hoarse voice and no change in NCV (14, 15). Traditional CMT classification cannot separate the forms of demyelinating, intermediate or axonal together and it depends on the clinical features of the patients. Recent studies have shown that GDAP1 is located on the outer mitochondrial membrane (16,17) and it is possible for GDAP1 protein to regulate in mitochondrial network dynamics and induce mitochondrial fragmentation without apoptosis mechanism (16). The first mutation in GDAP1 gene was found in the Mediterranean region (7, 8, 12, 18, 19). The 7 published GDAP1 frameshift mutations are listed in Table 1 (8, 9, 15, 20-22).

Here we report an Iranian patient with homozygote insertion in exon 1 (c.100_101insT) which is predicted to create an early stop codon interrupting the protein at codon 46. His parents were heterozygote in this position. This insertion was not reported previously and was not found in our control.

The N terminal part of the GDAP1 protein containing GST-N and GST-C domains is oriented toward the cytosol (16). Studies of GDAP1 point mutation in highly conserved amino acids of the GST-N domain (Y29A, S34A, S36A, S37A and F68S) demonstrate a close relation between GDAP1-induced mitochondrial fission and its GSH conjugation activity. In all of these point mutations, especially in S34A mutation, increasing the number of mitochondrial aggregates that accumulated in perinuclear regions was seen (23). Because novel mutation in the GST-N domain as a frameshift (c.100_101insT) may be associated with a common haplotype, suggesting a common ancestor that needs further investigation in the Iranian population.

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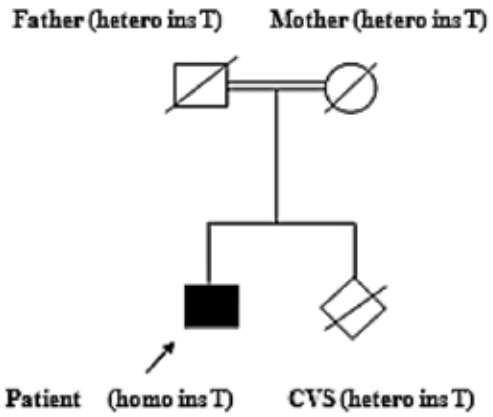

Fig 1. Pedigrees with a proven autosomal-recessive form of Charcot-Marie-Tooth (CMT4A)

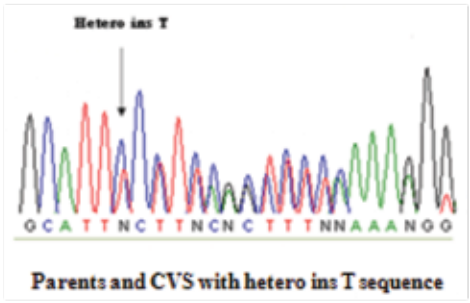

Fig 2. Novel T nucleotide insertion (C: 34) in the first exon of GDAP1 gene

Table 1. Summary of 7 Reported Frameshift Mutations of GDAP1 Gene

Framshift Mutations		Alias	Phenotype	Reference
Insertion	c.[349_350insT]+[349_350insT]	[Tyr117fs]+[Tyr117fs]	CMT4A	18
	c.[487C>T]+[863insA]	[Gln163X]+[Thr288fs]	CMT2 with vocal cord paresis	8
	c.[100_101insT]+[100_101insT]	[Ser34fs]+[Gln46X]	CMT4A	This study
Deletion	c.[341_344delAAAG]+[715C>T]	[Glu114fs]+[Leu239Phe]	CMT4A	22
	c.[439delA]+[439delA]	[Thr147fs]+[Thr147fs]	CMT4A	21
	c.[558delT]+[558delT]	[Ile186fs]+[Ile186fs]	CMT2 + vocal cord and diaphragm paralysis	15
	c.[786delG]+[786delG]	[Gly262fs]+[Gly262fs]	CMT4A	9
	c.[341_344delAAAG]+[487C>T]	[Glu114fs]+[Gln163X]	CMT2	23

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