An Algorithm for Genetic Testing of Serbian Patients with Demyelinating Charcot-Marie-Tooth

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Charcot-Marie Tooth (CMT) is a clinically and genetically heterogeneous group of diseases with rough genotype–phenotype correlation, so the final diagnosis requires extensive clinical and electrophysiological examination, family data, and gene mutation analysis. Although there is a common pattern of genetic basis of CMT, there could be some population differences that should be taken into account to facilitate analyses. Here we present the algorithm for genetic testing in Serbian patients with demyelinating CMT, based on their genetic specificities: in cases of no *PMP22* duplication, and if X-linked CMT (CMTX) is not contraindicated by pattern of inheritance (male-to-male transmission), one should test for c.94A>G *GJB1* founder mutation, first. Also, when a patient is of Romani ethnicity, or if there is an autosomal recessive inheritance in a family and unclear ethnicity, c.442C>T mutation in *NDRG1* should be tested.

Introduction

HEREDITARY MOTOR AND sensory neuropathies, also called Charcot-Marie-Tooth (CMT) disease, comprise a group of inherited peripheral neuropathies. CMT affects one in 2,500 people (Skre, 1974) and is the most common inherited neurological disorder (Kedlaya, 2012).

Demyelinating CMT or CMT1, the most common form of the disease, accounts for more than 60% CMT cases (Numakura *et al.*, 2003), and is characterized by lowered nerve conduction velocity (NCV) both in sensory and motor nerves. The other major form is axonal CMT or CMT2, distinguished by normal or mildly reduced NCVs.

CMT is a clinically and genetically heterogeneous group of diseases with rough genotype–phenotype correlation, so the final diagnosis requires extensive clinical and electrophysiological examination, family data, and gene mutation analysis. To date, about 30 genes and 50 loci have been associated with CMT (Inherited Peripheral Neuropathies Mutation Database-IPNMD, 2011). Among them, mutations in the *PMP22*, *MPZ*, and *GJB1* genes are responsible for the vast majority of patients with CMT, while mutations in other genes are often present in rare, even individual cases (Mersianova *et al.*, 2000; Mostacciolo *et al.*, 2001; Numakura *et al.*, 2002; Keckarevic Markovic *et al.*, 2009).

PMP22 and *MPZ* are associated with autosomal dominant forms of CMT1 (and CMT2 for *MPZ*), while *GJB1* is responsible for X-linked CMT (IPNMD, 2011). Autosomal recessive (AR) forms are rare, but could be over-represented in small

populations, due to a founder effect. Therefore, a founder mutation in the *NDRG1* gene is responsible for CMT1 ARD (CMT4D), also known as CMT Lom, the most common peripheral neuropathy among Romani people (Kalaydieva, 2002).

The genetic basis of CMT varies among populations, but the pattern is the same: the most frequent mutation in CMT1 is *PMP22* duplication, then *GJB1*, *MPZ*, and *PMP22* mutations. For CMT2, the gene mostly affected by mutations is *MFN2*, then *GJB1*, and *MPZ*. Therefore, the main algorithm for genetic testing of patients with CMT worldwide would be the same, suggesting genetic analyses in the order mentioned above. *GJB1* gene analyses should not be done if there is male-to-male transmission of the disease.

Despite that, in general, the given algorithm is the best way to genetically evaluate most CMT cases, there could be some population differences that should be taken into account to facilitate analyses, to make them faster, cheaper, and easier.

Here, we present the algorithm for genetic testing in Serbian patients with demyelinating CMT, based on their genetic specificities.

Material and Methods

Patient samples

A total of 98 patients with unrelated demyelinating CMT, 23 family members and 100 healthy controls participated in this study. CMT was diagnosed in patients based on the clinical and electrophysiological findings: distal muscle

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TABLE 1. RESULTS OF THE MUTATION ANALYSIS IN SERBIAN PATIENTS WITH DEMYELINATING CHARCOT-MARIE-TOOTH

MUTATION	PMP22 duplication	GJB1 total	GJB1 c.94A>G	MPZ	PMP22	EGR2	LITAF/SIMPLE	NDGR1 c.442C>T	SUM
(%)	37.7	10.2	5.1 ^a	3.1	1.0	0	0	5.1	57.1

^aNot included in a summary of percentages.

Numbers indicate the percentage of a specific mutation and/or a gene.

weakness and atrophy, absent or reduced deep tendon reflexes, sensory loss, and reduced NCVs. The study was approved by the Ethics Committee of the Institute for Neurology and Psychiatry for Children and Youth, and informed consents were obtained.

DNA isolation, mutation screening, and haplotype analysis

DNA was isolated from blood samples and buccal swabs. 17p11.2 duplication/deletion screening was performed by polymerase chain reaction (PCR)/*EcoRI* restriction digestion (Stronach *et al.*, 1999) and by microsatellite analysis using six polymorphic markers located in the involved region (Mersianova *et al.*, 2000). The coding regions, exon-adjacent sequences, and some promoters of *GJB1*, *MPZ*, *PMP22*, *EGR2*, and *LITAF/SIMPLE* were amplified using PCR, and then sequenced. The primer sequences are available upon request. Sequencing variations were confirmed by sequencing both strands. *NDGR1* c.442C>T mutation detection was per-

formed by PCR/*TaqI* restriction digestion (Kalaydjieva *et al.*, 2000).

Microsatellite markers surrounding *GJB1* were analyzed using fluorescent-labeled primers. The marker positions and primers are available upon request.

Results

The *PMP22* duplication was detected in 37/98 patients. Six different *GJB* mutations were detected in 10/98 patients, five of them detected for the first time in the Serbian population. Haplotype analysis showed that one of them, c.94A>G, present in five patients, was a founder mutation. Three mutations were found in *MPZ* (one identified for the first time), one in *PMP22*, while no mutations were found in *EGR2* and *LITAF/SIMPLE*. Mutation c.442C>T in *NDGR1* was identified in 5/98 patients, all of them were of Romani descent.

Results of mutation analysis are shown in Table 1.

FIG. 1. An algorithm for genetic testing of patients with demyelinating Charcot-Marie-Tooth (CMT) in a population of Serbia.

According to the results obtained, an algorithm for genetic testing of patients with demyelinating CMT in a population of Serbia was constructed, as shown in Figure 1.

Discussion

The results of genetic analyses in Serbian patients with CMT showed no major differences compared to other populations studied earlier (Keckarevic-Markovic *et al.*, 2009). *PMP22* duplication was found to be the most frequent CMT mutation, followed by *GJB1*, *MPZ*, and *PMP22* mutations. *EGR2* and *LITAF/SIMPLE* mutations were not found. However, six out of 10 mutations identified in this study have never been reported before. A large contribution of newly identified mutations confirmed the necessity for sequencing analyses of gene candidates, which is often time consuming and expensive.

One newly identified mutation in *GJB1*, c.94A>G, was found to be a founder mutation, and is present in 50% of patients with genetically confirmed CMTX, with overall contribution of 5.1%. Also, there are about 400,000–500,000 gypsies in Serbia (around 5% population), and CMT4D is the most frequent peripheral neuropathy among them, due to a c.442C>T founder mutation in *NDRG1*. In the Serbian CMT population, CMT4D accounts for another 5.1%. In both cases, restriction digestion analysis or allele-specific PCR, fast and inexpensive compared to gene sequencing, should be the methods of choice.

An algorithm for genetic testing of patients with demyelinating CMT in a population of Serbia was constructed regarding a respectable proportion of Serbian patients with CMT (~3,6%), considering that there are 7,000,000 people in Serbia and that the estimated frequency of CMT is 1 in 2,500. According to a given guideline, in cases of no *PMP22* duplication, and if CMTX is not contraindicated by the pattern of inheritance (male-to-male transmission), one should test for the c.94A>G *GJB1* mutation, first (Fig. 1). No, especially, frequent mutation was outlined in the guidelines for genetic testing by Saporta *et al.* (2011) and Murphy *et al.* (2012), implicating that the high frequency of that c.94A>G *GJB1* mutation is a consequence of a genetic homogeneity of the Serbian population.

Also, when a patient is of Romani ethnicity, or if there is an AR inheritance in a family and unclear ethnicity, a c.442C>T mutation in *NDRG1* should be tested. Compared to other published algorithms (Saporta *et al.*, 2011; Murphy *et al.*, 2012) in Serbian patients with CMT, a greater percentage of genetically confirmed AR CMT is expected, due to a large proportion of Romani people in Serbia, bearing a founder recessive mutation in the *NDRG1* gene combined with consanguinity.

The given algorithm should provide an overall guidance to genetic testing of Serbian patients with CMT with lowered nerve conduction velocities. In cases of specific information, the order of analyses could be changed and adjusted to a specific situation.

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Authors Disclosure Statement

No competing financial interests exist.

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