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CMT4G – Rare Form of Charcot-Marie-Tooth Disease in Slovak Roma Patient

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***Abstract*—**The Roma (Gypsies) is a transnational minority with a high degree of consanguineous marriages. Similar to other genetically isolated founder populations, the Roma harbor a number of unique or rare genetic disorders. This paper discusses about a rare form of Charcot-Marie-Tooth disease – type 4G (CMT4G), also called Hereditary Motor and Sensory Neuropathy type Russe, an autosomal recessive disease caused by mutation private to Roma characterized by abnormally increased density of non-myelinated axons. CMT4G was originally found in Bulgarian Roma and in 2009 two putative causative mutations in the *HK1* gene were identified. Since then, several cases were reported in Roma families mainly from Bulgaria and Spain. Here we present a Slovak Roma family in which CMT4G was diagnosed on the basis of clinical examination and genetic testing. This case is a further proof of the role of the *HK1* gene in pathogenesis of the disease. It confirms that mutation in the *HK1* gene is a common cause of autosomal recessive CMT disease in Roma and should be considered as a common part of a diagnostic procedure.

***Keywords*—**Gypsies, *HK1*, HSMN-Russe, rare disease.

I. Introduction

COMPLEX and very specific structure of Roma (Gypsy) society has long attracted the attention of cultural anthropologists. It was not always so in the field of human genetics. Unlike other founder populations Roma have been ignored by European medicine for hundreds of years, and their unique genetic heritage is only recently becoming a focus of interest for geneticists and medical practitioners [1]. Roma ethnic group represents a very unique population, formed of socially and culturally heterogeneous and geographically dispersed subisolates. Endogamy and a high degree of consanguinity are characteristic for Roma groups. These features, together with the fact all European Roma are descendants of a small group of ancestors that left India about 1000 years ago, makes the Roma communities a valuable target for geneticists. Roma often suffer from diseases that are common in the surrounding populations, but in Roma they are caused by a different mutation. Many private mutations and single gene disorders were revealed in the last decades [1]–[3].

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The molecular cause of number of diseases in the Roma population has been clarified in the past years [4]-[6]. Due to founder effect, many of mutations causing these diseases are exclusive for Roma and identical in all affected families of all Roma populations. It is highly expected that these conditions are present in Slovak Roma population as well. The population of Roma in Slovakia is one of the largest in Europe, outnumbered only by Roma communities in Romania, Bulgaria and Hungary. The estimated number of Roma in Slovakia is 400.000 [7].

Genetic studies in the past two decades have brought a lot of knowledge about origin, migration a structure of Roma groups in Europe. First of all, they provided final proof Roma originate from India – the theory that had been accepted by most scientists but missed the physical evidence to become the scientific fact [1]-[3], [8]. Almost 45% of Y chromosomes in studied Roma population belong to VI-68 haplogroup [3], which is completely absent in European chromosomes [9]. mtDNA haplogroup M, that is specific for Asian populations, accounts for 26.5% of maternal lineages in Roma [3]. Detailed analyses of haplotypes shed a light on circumstances of departure from India: the time of Roma exodus from India dates to 970–1170 years ago, there is evidence that all present Roma groups are of common descend, that the group that left India was very small, consisting of around 1000 people, and derived from distinct caste or tribal group [1]-[3], [5], [10]. Analysis of microsatellite markers on Y chromosomes, in mtDNA and in the close proximity of mutations private to Roma estimates the period when the migrating population first split into small groups to be around thirteenth century [2], [10], [11]. Roma groups in Europe are nowadays separated by large genetic distances [3].

Charcot-Marie-Tooth (CMT) hereditary neuropathy is heterogeneous group of disorders characterized by a chronic motor and sensory polyneuropathy. The affected individual typically has distal muscle weakness and atrophy often associated with mild to moderate sensory loss, depressed tendon reflexes and high-arched feet [12]. CMT is classified into numerous types that differs in mode of inheritance and electrophysiological or anatomical pathology findings (axonal or demyelinating), but many types can be distinguished only on the basis of molecular genetic analysis [13]. Majority of CMT types is inherited in autosomal dominant or X linked manner. Autosomal recessive forms of CMT (ARCMT) are very rare; they have earlier onset and more severe phenotype [14]. Axonal forms of ARCMT belong to the CMT2 group, while demyelinating forms constitute a separate group named CMT4. Eleven genes causing demyelinating ARCMT have

been identified (some of them very recently), each causing a subtype named CMT4A–J [15], [16]. ARCMT account for less than 10% of the families in the European CMT population but can account for 30%–50% of all CMT cases in the populations with high prevalence of consanguineous marriages, typically in the Mediterranean basin and the Middle East but also in Roma/Gypsy communities in Europe [17], [18]. There are three subtypes of autosomal recessive CMT with mutations private to Roma population – CMT4C, CMT4D and CMT4G [14], [19], [20]. The most common type is CMT4D (MIM 601455), also known as hereditary motor and sensory neuropathy type Lom (HMSNLom). The mutation associated with the disease, p.R148X in the *NDRG1* gene, is found almost exclusively in Roma populations [2], [5] (MIM605285), also known as hereditary motor and sensory neuropathy type Russe (HMSN-Russe) was originally described in Bulgarian Roma [14], [21]. A mutation in an alternative untranslated exon of the hexokinase 1 (*HK1*) gene, later denoted as g.9712G>C (NM_033498) [22], was associated with the disease in 2009 [6]. CMT4C (MIM 601596) is caused by a spectrum of mutations in the SH3TC2 gene. However, mutation p.R1109X in the SH3TC2 gene associated with a conserved haplotype is considered to be private for Roma and it is a predominant cause of CMT4 in Spanish Roma [20], [22], [23].

ARCMT can be properly diagnosed only by genetic analysis of responsible genes. However, due to rarity of this diseases and their absence in the majority (non-Roma) population in Slovakia, genetic analysis of ARCMT is not a part of diagnostic procedure and patients are left only with general diagnosis of sensorimotor neuropathy of unspecified type. We have recently performed a genetic analysis in four Slovak Roma families with such unspecific diagnosis, with autosomal recessive inheritance and demyelinating type of neuropathy. Based on sequencing results we diagnosed CMT4D or CMT4G in 100% of examined families [24].

Here we present the third case of CMT4G reported in Slovakia, which confirms that mutations in the *HK1* (hexokinase 1) gene are a common cause of ARCMT disease not only in Roma populations of Bulgaria and Spain, as reported before [14], [22], but also in Slovakia and presumably in other European Roma communities.

II. Material and Methods

The study was conducted after obtaining written informed consent from the proband in accordance with the ethical principles based on the Declaration of Helsinki. Proband was a 21-year old woman from Eastern Slovakia. She identified herself and her family as Roma (Gypsy). Consanguinity in family was denied. No other family member including 19-year old sibling was affected. The proband has a one year old son, who was asymptomatic, however, not available for examination.

Genomic DNA was isolated from peripheral white blood cells using a blood DNA isolation kit (MO BIO Laboratories Inc). We sequenced three regions: exon 7 of the *NDRG1* gene, a region surrounding G>C substitution in the AltT2 exon of the *HK1* gene [6], later named g.9712G>C (NM_033498) [22] and a region surrounding intronic G>A substitution 1315 bp downstream of the AltT2 change in the *HK1* gene [6] named g.11027G>A [24]. Following the PCR amplification, sequencing was performed using a terminator kit BigDye version 3.1 (Life Technologies) on an automated sequencer 3500 Genetic Analyzer (Life Technologies). Same primers were used for amplification and sequencing reaction. Primer sequences are shown in Table I.

TABLE I
Sequences of Primers

Gene	Region	Variant	Primer sequence	Product size (bp)
NDRG1	exon 7	g.631C>T	F: gggaagaaatgatgccgtaa	575
			R: aagcccatgacaaaggaatg	
HK1	AltT2 exon	g.9712G>C	F: ggaaatccatctctcctctcaa	570
			R: tcaccccacaataaaaactcg	
HK1	downstream of AltT2 exon	g.11027G>A	F: gcctgggtcacagttaggaa	336
			R: tctgagatgcaatctacctcca	

F – forward primer, R – reverse primer

III. Results and Discussion

In this study we performed genetic examination of a proband of Roma origin in Slovakia with early-onset demyelinating neuropathy and autosomal recessive inheritance. The proband displayed early onset demyelinating neuropathy. First symptom was described as a pain in the calves at the age of two, followed by frequent falls and gait difficulties. Patient was monitored by neurologist from the age of 9. Clinical examination and EMG shows demyelinating peripheral neuropathy with moderately reduced motor nerve conduction velocities. Apart from impaired walking, the proband reported no other subjective difficulties at the time of examination.

The regions spanning three variants in *NDRG1* and *HK1* genes previously associated with CMT4D and CMT4G, respectively, were sequenced. We detected variant g.9712G>C (Fig. 1) and variant g.11027G>A (Fig. 2) in *HK1* gene in homozygous form in proband. No other family members were available for analysis. Based on this information, patient was diagnosed with Charcot Marie-Tooth type 4G disease. The characteristic feature of CMT4G neuropathology is the profuse regenerative activity with numerous clusters of thinly myelinated regenerating fibers leading to an abnormally increased density of non-myelinated axons. CMT4G is characterized by delayed early motor development, and steady progression to total muscle paralysis. Sensory loss is prominent and affects all modalities. Motor nerve conduction velocities (MNCV) are reduced in the intermediate range already in the second half of the first decade of life [6], [14], [25]. CMT4G was first mapped to 10q23 in 2000 [21]. In 2009 [6] identified two putative causative mutations in the *HK1* gene: G>C in an alternative untranslated exon (AltT2) and G>A in the adjacent intron.

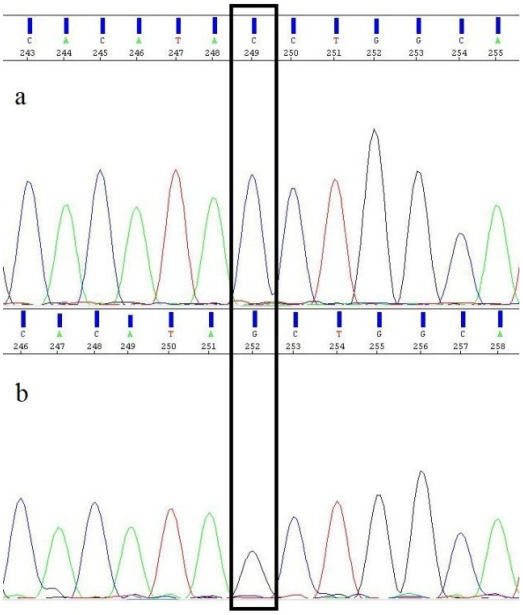

Fig. 1 Chromatographs of mutation g.9712G>C. (a) homozygous substitution in the proband, (b) wild-type genotype in a control sample

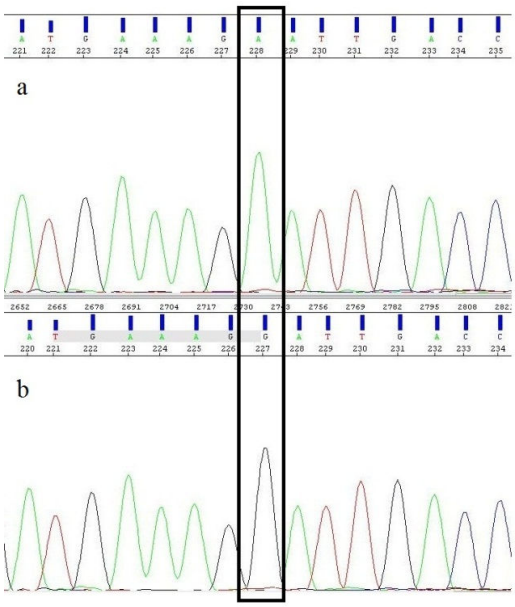

Fig. 2 Chromatographs of mutation g.11027G>A. (a) homozygous substitution in the proband, (b) wild-type genotype in a control sample

Sequence conservation of the AltT2 exon and analysis of the *Hk1* upstream region in mouse mRNA strongly favor the exonic change as the pathogenic mutation, however, both variants are in complete linkage disequilibrium and segregate with the disease in all affected families. Hexokinases phosphorylate glucose to produce glucose-6-phosphate, the first step in most glucose metabolism pathways and serves as a major point of regulation of the energy-producing glycolytic pathway. *HK1* gene encodes a ubiquitous form of hexokinase which localizes to the outer membrane of mitochondria. *HK1* is ubiquitously expressed and particularly abundant in the brain, the testis and erythrocytes [6]. Our study confirms a role of one of putative *HK1* mutations in pathophysiology of CMT4G, which has not yet been confirmed by functional studies.

Several mutations specific for the Roma population have been revealed in the past several years. It is necessary for clinicians to obtain a good knowledge of the conditions specific for ethnic groups and to focus diagnostic procedure on mutations specific for these groups. Otherwise, an underdiagnosis or wrong diagnosis of these rare conditions may remain a problem. The situation with the Roma ethnicity is even more complicated by the fact that their low education, social status and economic situation are the reason that they would often not attend further examinations, leaving physicians with the lack of information for proper diagnosis.

The case of CMT4G reported here is a proof that this disease is a common type of ARCMT among Slovak Roma. Knowledge of the diseases and particular mutations specific to a certain ethnic group can greatly simplify and speed up the diagnostic process. CMT4G is a rare form of CMT, which should considered for molecular analysis in patients of Roma origin with demyelinating type of neuropathy. Genetic analysis of the *HK1* gene should be considered as a common part of diagnostic procedure since it would contribute to clarifying complicatedly diagnosable cases of demyelinating neuropathy in countries with Roma minority.

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