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Short Report

HSMNR belongs to the most frequent types of hereditary neuropathy in the Czech Republic and is twice more frequent than HMSNL

Šafka Brožková D., Haberlová J., Mazanec R., Laštůvková J., Seeman P. HSMNR belongs to the most frequent types of hereditary neuropathy in the Czech Republic and is twice more frequent than HMSNL.
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Hereditary motor and sensory neuropathy type Russe (HMSNR), also called CMT4G, is an autosomal recessive inherited peripheral neuropathy (IPN) caused by a founder mutation in the *HK1* gene. HMSNR affects only patients with Roma origin, similar to the better known HMSN type Lom clarified earlier. By testing IPN patients with Roma origin, we realized that HMSNR affects surprisingly many patients in the Czech Republic. HMSNR is one of the most frequent types of IPN in this country and appears to be twice more frequent than HMSNL. Pronounced lower limb atrophies and severe deformities often lead to walking inability in even young patients, but hands are usually only mildly affected even after many years of disease duration. The group of 20 patients with HMSNR presented here is the first report about the prevalence of HMSNR from central Europe.

Conflict of interest

The authors declare no conflict of interest.

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Inherited peripheral neuropathy (IPN) is a genetically highly heterogeneous group of disorders consisting of a large number of neuropathies depending on the type of neuropathy and inheritance.

The Roma are a significant ethnic minority (150,000–250,000 individuals) in the Czech Republic, originally having migrated from India (1, 2). Roma form a genetically isolated group with high endogamy (3). Because of these characteristics, Roma have specific hereditary diseases mostly caused by specific recessive mutations. These apply also for IPN; two types of non-syndromic hereditary neuropathy are found only in Roma; HMSNL (CMT4D, OMIM 601455), Hereditary motor and sensory neuropathy type Russe (HMSNR) (CMT4G, OMIM 605285)(4–6). Only one homozygous mutation in each type is responsible for the disease; *NDRG1* (NM_006096.3, p.R148*, c.422C>T) for HMSNL, *HK1* (NM_033498.2, g.9712G>C (c.-249-3818G>C) for HMSNR (7, 8).

The HMSNR phenotype was first reported in one branch of an extended Bulgarian family in 2000 (5) and it took another 9 years to discover the molecular basis for

HMSNR and allow a precise diagnostics (8). This may be the reason why HMSNR is less known and why little has been published about it. So far, HMSNR patients have been reported from only two other countries aside from Bulgaria (9–11). The initial report described 34 HMSNR patients, mainly from Bulgaria, and also stated the heterozygous carrier rate among Roma to be 5 in 790 (8). The report from Spain described the HMSNR as the second most frequent type of IPN among 29 Roma families (9). The latest paper about HMSNR reports two families from Slovakia, but no other data about the frequency was provided, nor was a larger group of examined patients studied (10).

The prevalence of HMSNR and representation to the other types of IPN is largely unknown. Prior to our study, no patients from the Czech Republic nor from any other central European country were reported except the two Slovak patients.

Here, we show that HMSNR belongs to the most frequent types of IPN in the Czech Republic and we report findings in 20 HMSNR patients. This cohort is the largest with HMSNR after the original description.

Materials and methods

Patients

In total, 74 patients were examined, namely 32 Roma patients, 18 Roma individuals with HMSNL mutation and 24 patients with no data on ethnicity (thereof 15 with AR IPN, previously tested negative with custom panel designed to test all IPN genes (HaloPlex, Agilent technologies – targeted massively parallel sequencing). Patients were collected from all of the Czech Republic as our laboratory is the only one for DNA testing of IPN. Homozygotes or heterozygotes (10 + 8) for HMSNL mutation (*NDRG1*; NM_006096.3; p.R148*, c.422C>T) were examined in order to exclude possible double carrier of HMSNL and HMSNR as was previously described in one family (12). All examined persons signed informed consent with DNA examination approved by the local ethics committee.

Clinical and electrophysiological examination

Eleven HMSNR patients were re-examined by physicians from our IPN group (6-P.S., 3-J.H., 2-R.M.), nine were examined by attending physicians and were not willing for re-examination. Electrophysiological examinations were performed in different laboratories therefore the final decision is reported in Table 1 and nerve conduction studies (NCS) in six patients examined in our department are in Appendix S1, Supporting information.

Sequencing

Genomic DNA was examined for two variants in *HK1* (RefSeq NM_033498.2). First, g.9712G>C variant (c.-249-3818G>C, rs397514654) and second, G>A substitution 1315 bp downstream of the first variant, namely the g.11027G>A (c.-249-2503G>A, rs545102911). Primers were designed with primer Blast for the 150 bp region from both sides of the variants (13). PCR fragments were sequenced using BigDye Terminator v3.1 and separated on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence chromatograms were analyzed by Mutation Surveyor program (Softgenetics, State College, PA, USA).

Results

Twenty patients from 16 independent families were found to be homozygous for the c.-249-3818G>C (g.9712G>C) variant in *HK1*. The second variant c.-249-2503G>A (g.11027G>A) was found also homozygous in all patients and heterozygous in 10 carriers of the g.9712 variant. One patient tested negative for g.9712G>C variant was found to be a carrier of heterozygous g.11027G>A variant.

Clinical findings are summarized in Table 1. Photos are shown in Figure 1. The onset of the disease ranged from 3 to 15 years of age (average 7.5 years). Hands are affected later in the third decade and are only mildly affected compared with feet and legs. Five patients have complete paralysis of distal muscles combined with proximal weakness on the lower limbs (Table 1 – patients 1, 13, 15, 17, 18). The three oldest patients were 39, 44, and 46 years old and have only mild or no distal weakness and atrophies of the upper limbs (Table 1 – patients 7A, 11C, 12C). Almost all patients suffer from foot deformity, frequently severe, and foot surgery was frequently performed. Two patients have been confined to a wheel chair after 12 and 13 years of disease duration (Table 1 – patients 1 and 17). Scoliosis was found only in one patient (Table 1 – patient 6). Vibration sense loss was detectable in all examined patients. Pinprick and touch sensitivity are missing in some patients.

Conclusion of NCS showed 14 patients with demyelinating and 4 with intermediate neuropathy. Even so, NCV in patients examined in our laboratory were mostly in the intermediate range between 30–38 m/s for motor and 24–34 m/s for sensory nerves, but the compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) amplitudes were severely decreased as the sign of pronounced axonal damage or loss (Appendix S1).

Two siblings are affected in families A, C and D. The uncle and nephew are affected in family B (Appendix S1).The family A was detected by chance; the coding region of *HK1*, but not the deeply intronic region with the HMSNR mutation site, was included in the gene panel examined by massively parallel sequencing.

Therefore, we examined the *HK1* mutation by Sanger sequencing in patients without dominant inheritance, previously tested negative by this panel. This testing revealed the HMSNR in family A. Subsequent targeted questioning revealed the same family name of mother (maiden name), father (the grandparents were from the same village in Slovakia) and Roma origin. Patient 18 was adopted, therefore the type of inheritance was

Table 1. Clinical findings in Czech HMSNR patients.

Patient/ family[a]	Onset LL/ UL (years)	Disease duration/age (years)	Weakness LL/UL	Atrophies LL/UL	Sensory loss	Deformities	Other	NCS
1	11/–	16/27	LL 4–5/0 UL 5/4	LL +++ UL +	V, T	Pes equinovarus	Wheel chair (since 23 years)	CMT1
2	3.5/no	Several months/3.5	Unstable gait	–	–	Pes valgus	No	CMT1
3	2/–	18/20	LL 5/0 UL 5/3	LL ++ UL +	V, T	Pes equinovarus	Foot surgery	CMT1
4	7/no	3/10	LL 5/2 UL 5/5	LL ++ UL no	V	Pes cavus	No	CMT1
5	6/no	Several months/6	–	LL ++ UL –	–	–	No	CMT1
6	4.5/–	7.5/12	Unstable gait	LL ++ UL ++	–	Pes equinovarus	Foot surgery, scoliosis	CMT1
7/A	8/25	36/44	LL 5/0 UL 5/4+	LL ++ UL no	V	Mild pes cavus	Walk with crutches and orthesis	CMT1
8/A	7/–	22/29	LL 5/0 UL 4/4	LL ++ UL no	V	Pes cavus	Walk with crutches and orthesis	CMTInt.
9/B	10/23	14/24	LL 5/2 UL 5/4	LL +++ UL +	V, T, P	Pes equinovarus	Foot surgery	CMT1
10/B	12/–	25/37	Unstable gait	LL – UL +	–	Pes cavus	–	CMT1
11/C	3/–	36/39	LL 5/0 UL 5/5	–	V	Pes cavus	Foot surgery	CMT1
12/C	12/–	34/46	–	LL ++ UL +	–	Pes cavus	–	CMTInt.
13/D	8/30	25/33	LL 4/0 UL 5/4	LL +++ UL ++	V, T, P	Pes equinovarus	Foot surgery	CMT1
14	0–5/–	–/14	– UL 5/5	– UL no	–	Pes cavus	–	CMTInt.
15	–/–	–/44	LL 4/0 UL –	LL –. UL +	–	–	–	CMT1
16	5–10/–	–/14	LL 5/4 UL 5/5	LL ++ UL no	–	Pes cavus	Foot surgery	CMT1
17	15/–	25/40	LL 4/0 UL 5/5	LL +++ UL no	V, T	Pes equinovarus	Wheel chair (since 28 years)	CMTInt.
18	3/14	20/23	LL 4/0 UL 5/3+	LL ++ UL ++	V	Pes equinovarus	Foot surgery	CMT1

Weakness based on MRC scale for proximal and distal segment of the lower (LL) and upper (UL) limbs, proximal/distal; atrophies LL + = feet, LL ++ = below knee, LL +++ = above knee, UL + = hand, UL ++ = forearm, UL +++ = above elbow; V = vibratory, T = touch, P = pinprick; NCS = nerve conduction study; CMT1 = demyelinating neuropathy, CMTInt. = intermediate neuropathy; – = no data.

[a]Two patients are missing in Table 1, no data are available for them.

unknown and DNA testing of frequent types of IPN was negative. Only the additional information about the Roma origin, added 14 years later, lead us to the correct DNA testing and clarification of IPN.

HMSNR patients form about 1.1% of all genetically clarified IPN patients in the Czech Republic because our laboratory is the only laboratory performing DNA testing for IPN. From the perspective of the families, it is 1.7% of HMSNR families in a group of all the IPN families with genetic diagnosis. It appears that at least in the Czech Republic, HMSNR is twice more frequent than HMSNL, where the examination of the same cohort revealed only 10 homozygotes in seven families (Table 2).

Discussion

The detected 20 patients with homozygous founder HK1 mutation was an unexpected surprise because only a few patients have been reported outside of Bulgaria to date. Two variants in *HK1* (c.-249-3818G>C and

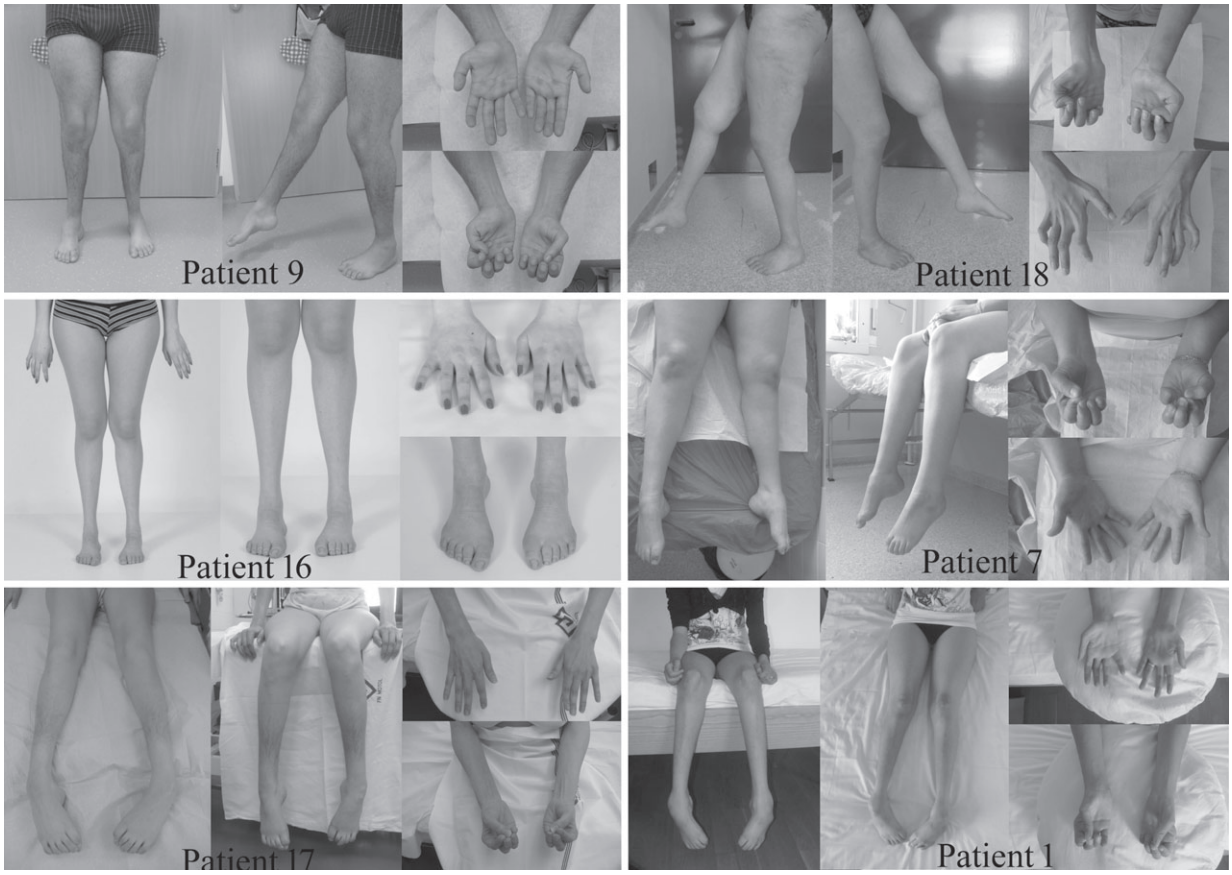

Fig. 1. Pictures of HMSNR selected patients – distal plegia and atrophies of lower limbs are shown in all patients except the 14 years old patient 16. Upper limbs are usually only mildly affected in all the patients. Only patient 9 reported problems with hands started year ago and patient 18 has pronounced atrophies, contractures and problems with buttons, zippers (affection of hands has lasted for 14 years).

Table 2. Number of Czech IPN patients and families with causal mutations according to mutated genes.

Gene	Patients (%)	Families (%)
All	1747	944
1. CMT1A/HNPP	1245 (71.3)	685 (72.6)
2. GJB1	191 (10.9)	74 (7.8)
3. MPZ	64 (3.7)	34 (3.6)
4. MFN2	39 (2.2)	21 (2.2)
5. HINT1	23 (1.3)	21 (2.2)
6. **HK1 (HMSNR)**	20 (1.1)	16 (1.7)
6. SH3TC2	20 (1.1)	16 (1.7)
7. PMP22	22 (1.3)	15 (1.6)
8. NDRG (HMSNL)	10 (0.6)	7 (0.7)
9. GDAP1	9 (0.5)	7 (0.7)
10. BSCL2	12 (0.7)	5 (0.5)

The table is sorted according to a number of families with each causal mutation.

c.-249-2503G>A) are reported in HMSNR patients. Both variants were inherited together in our patients. Therefore it is not possible to decide which one is the pathogenic and which one is only variant inherited together. The presence of g.9712G>C in alternative untranslated exon 2 predisposes it to be pathogenic (12). Our finding of g.11027G>A in a heterozygous state combined with wild-type allele on the g.9712 position in a patient with IPN support the hypothesis that the g.9712G>C variant is mutated on the ancestral allele with the older g.11027G>A variant.

Comparison of the frequency of mutated genes placed *HK1* as the sixth most frequently mutated gene among all the Czech IPN patients (Table 2). The number of HMSNR patients is almost the same as are groups of patients with confirmed *SH3TC2* and *HINT1* mutations, the most frequent recessive IPN. Only the CMT1A duplication/Hereditary neuropathy with liability to pressure palsies (HNPP) deletion (71.3%), *GJB1* (10.9%), *MPZ* (3.7%) and *MFN2* (2.2%) mutations are more frequent, but the inheritance is autosomal dominant for these.

The situation is more straightforward among Czech Roma. HMSNR is the most frequent type responsible for 45% of Roma patients with IPN. HMSNL is the second most frequent, twice less frequent, and found to be mutated in 24% of Roma with IPN. Comparison with other countries is possible to only one similar report from Spain; Sevilla reported the involvement of the IPN genes in Spanish Roma as follows; first *SH3TC2* (CMT4C) 57.1%, second *HK1* 24.1% and third *NDRG1* with 17.2%

patients (9). The involvement of *SH3TC2* is not frequent in the Czech Roma and no Roma patients were found with biallelic mutations in this gene (14).

The number of Czech Roma is difficult to ascertain. The census in 2011 showed only 11,000 Roma (15), but other sources suggest it is much higher. Usually the figure is between 2% and 3% (150,000–250,000) of the Czech population (2, 3). Taking into account the total number of Roma, then the rough estimation based on 20 HMSNR patients shows the minimal prevalence of HMSNR is 1:10,000. In comparison with other Roma diseases, congenital cataracts, facial dysmorfism and demyelinating neuropathy (CCFDN), found in 10 Czech patients, HMSNR is two times more frequent. CCFDN is easily recognizable because of a congenital cataract.

The clinical picture is similar to previous reports. The lower number of patients with upper limb affection could be because of a higher representation of young patients. The affection of the upper limbs starts between 10–43 years in Balkan Roma and 10–30 in Spanish patients (9, 16). In all the patients, except the young ones where the disease duration was several months to 3 years (Table 1 – patients 2, 4, 5), severe weakness and atrophies of the lower limbs required foot surgery, use of orthotics and often concluded in a wheel chair. Significant walking problems start in the third and fourth decades. The NCV in our HMSNR patients are decreased to 24–38 m/s in contrast to HMSNL patients with demyelinating neuropathy with very slow nerve conduction velocity (10–15 m/s) (17, 18).

No HMSNR carrier was found in individuals with HMSNL mutations. Moreover, Roma patients tested for HMSNR were previously tested negative for HMSNL. Therefore, the possible coincidence of occurrence of both variants proved to be very unlikely.

HMSNR appears to be the most frequent type of IPN in Czech Roma patients. The frequency may be similar in other central European countries. Our data show that it is rational in IPN patients of Roma ancestry to test first for the HMSNR founder mutation g.9712G>C and subsequently for the HMSNL founder mutation p.R148* and only if both tests are negative to continue to test for CMT1A/Hereditary neuropathy with liability to pressure palsies (HNPP). Information about ethnic origin is therefore crucial for effective and proper selection of the right DNA test. Examination of patients with recessive IPN should be considered where Roma origin may exist and where thus the HMSNR founder mutation could be inherited. The HMSNR founder mutation should be included in the panel for IPN testing. Therefore, it is useful to check the panel design whether the deeply intronic HMSNR mutation is included.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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