Hereditary motor and sensory neuropathy – Lom (HMSNL): refined genetic mapping in Romani (Gypsy) families from several European countries

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Abstract

Hereditary motor and sensory neuropathy type Lom, initially identified in Roma (Gypsy) families from Bulgaria, has been mapped to 8q24. Further refined mapping of the region has been undertaken on DNA from patients diagnosed across Europe. The refined map consists of 25 microsatellite markers over approximately 3 cM. In this collaborative study we have identified a number of historical recombinations resulting from the spread of the hereditary motor and sensory neuropathy type Lom gene through Europe with the migration and isolation of Gypsy groups. Recombination mapping and the minimal region of homozygosity reduced the original 3 cM hereditary motor and sensory neuropathy type Lom region to a critical interval of about 200 kb. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Hereditary motor and sensory neuropathy type Lom; Genetic mapping; Gypsy families

1. Introduction

Hereditary motor and sensory neuropathy type Lom (HMSNL) is a novel autosomal recessive demyelinating neuropathy associated with deafness, which was initially identified in Roma (Gypsy) families from Bulgaria [1,2]. The HMSNL gene has been mapped to 8q24 [1]. The conserved polymorphic haplotypes on 8q24, shared by all disease chromosomes in the original group of affected families, suggested genetic homogeneity and homozygosity for a single founder mutation. Since the initial description of the disorder, affected individuals have been diagnosed in

different countries across Europe, suggesting that HMSNL is probably the most common single autosomal recessive peripheral neuropathy.

The neuropathological features include a very early and severe axonal loss [1,3–7] pointing to HMSNL as a model disorder whose genetic and pathophysiological mechanisms may be relevant to understanding the general causes of disability in demyelinating peripheral neuropathies. Since neural deafness is an invariable symptom of HMSNL, the identification of the molecular basis of the disease will also contribute to disentangling the complexity of factors involved in hearing loss.

The HMSNL interval defined by the initial data spanned a distance of approximately 3 cM. The Src-like adaptor protein (SLAP), involved in receptor-mediated cell signalling, has been subsequently placed in that interval and excluded as the HMSNL gene [8]. Since no other candidate genes are known to be located in the region, the identification of the HMSNL gene has to rely on the positional cloning approach whose success depends on the size of the critical interval.

Here we present the results of a collaborative study of affected families diagnosed in Bulgaria, Spain, Italy, Slovenia, France, Germany and Rumania, aiming at the refined genetic mapping of HMSNL.

2. Patients and methods

2.1. HMSNL patients and families

The study comprised a total of 174 subjects: 60 patients and 114 unaffected relatives from 23 HMSNL families.

The group of patients included 32 males and 28 females aged between 7 and 50 years. Detailed clinical examinations were performed in all cases. Electrophysiological investigations, including nerve conduction studies and recordings of brainstem auditory evoked potentials (BAEPs) were carried out in at least one affected individual per family. Neuropathological studies were conducted on sural nerve biopsies obtained from seven unrelated affected subjects.

Informed consent for participation in the study has been obtained from all subjects. The study complies with the ethical guidelines of the institutions involved.

2.2. Methods

2.2.1. Polymorphic markers used in the study

A total of 32 markers on 8q24.3 were typed in the HMSNL families (Fig. 1). These included nine microsatellites flanking the HMSNL region, namely 41F08, D8S557, D8S1835 and D8S558 on the centromeric side, and D8S256, D8S1708, D8S1746, D8S1796 and D8S1462 on the telomeric side. A total of 23 markers were located in the interval between D8S558 and D8S256, defined in the initial study as the HMSNL gene region [1], Three of these microsatellites, namely D8S529, D8S378 and AFM116yh8 have been

published previously and are available through the public genome databases. The remaining 20 polymorphic microsatellites have been identified as part of this study.

2.2.2. Identification of new polymorphic microsatellites in the HMSNL region

A contig of genomic BAC or PAC clones spanning the HMSNL region was assembled and the clones were used for the identification of new polymorphisms. The BAC/PAC DNA was digested with frequent-cutter restriction endonucleases. The fragments were shotgun cloned into pBluescript. Colonies were gridded and a replica membrane was hybridized with a cocktail of [^{32}P]γ-ATP end-labelled di/tri/tetranucleotide repeat sequences. Alternatively, the fragments were ligated to adapter oligonucleotides RX24 (5$'$-AGCACTCTGCAGCCTCTAGATCTC-3$'$) and RX09 (5prime;-GAGATCTAG-3$'$), hybridized to a biotinylated anchor probe corresponding to a simple repeat sequence and captured using streptavidin-coated magnetic beads. The fragments were released by washing, PCR-amplified and cloned into pBluescript. In both cases the clones were sequenced, using universal primers T7 and T3, to identify the repeat sequence and select PCR primers for further analysis. Two of the new microsatellites were identified within BAC ends sequenced routinely as part of the physical mapping of the region, and three were found during the genomic sequencing of the HMSNL region.

All newly identified microsatellites were tested for polymorphism against a panel of DNA samples of unrelated control individuals from the same population and subsequently typed in the HMSNL families. Due to their low polymorphism, markers 423r133, 543b76, 4838-T3, 369CA2 and 369CA3 were analyzed only in the families with evidence of recombinations, in an attempt to map the breakpoints.

2.2.3. Genotyping analysis

Markers available through the public databases were PCR-amplified using commercially available fluorescently labelled primers (Research Genetics Map Pair Set). Electrophoretic length separation was carried out on an ABI 373 XL automated DNA analyzer. Allele assignment was performed using the ABI Genotyper software and alleles named by their size in base pairs.

The newly identified microsatellite repeats were analyzed through the incorporation of [^{32}P]α-dCTP in the PCR product during amplification. The PCR primers, the average length of the PCR products and the number of alleles are shown in Table 1. The PCR products were separated by vertical acrylamide gel electrophoresis at 1400 V for 1.5–2.5 h in a Hoefer Pokerface II apparatus. The gels were fixed in a 10% methanol/10% acetic acid solution, dried in a Savant slab gel dryer and exposed to Cronex 4 films for 2–24 h. Allele calling was performed manually, numbering the alleles from top to bottom. Uniform allele assignment across the sample
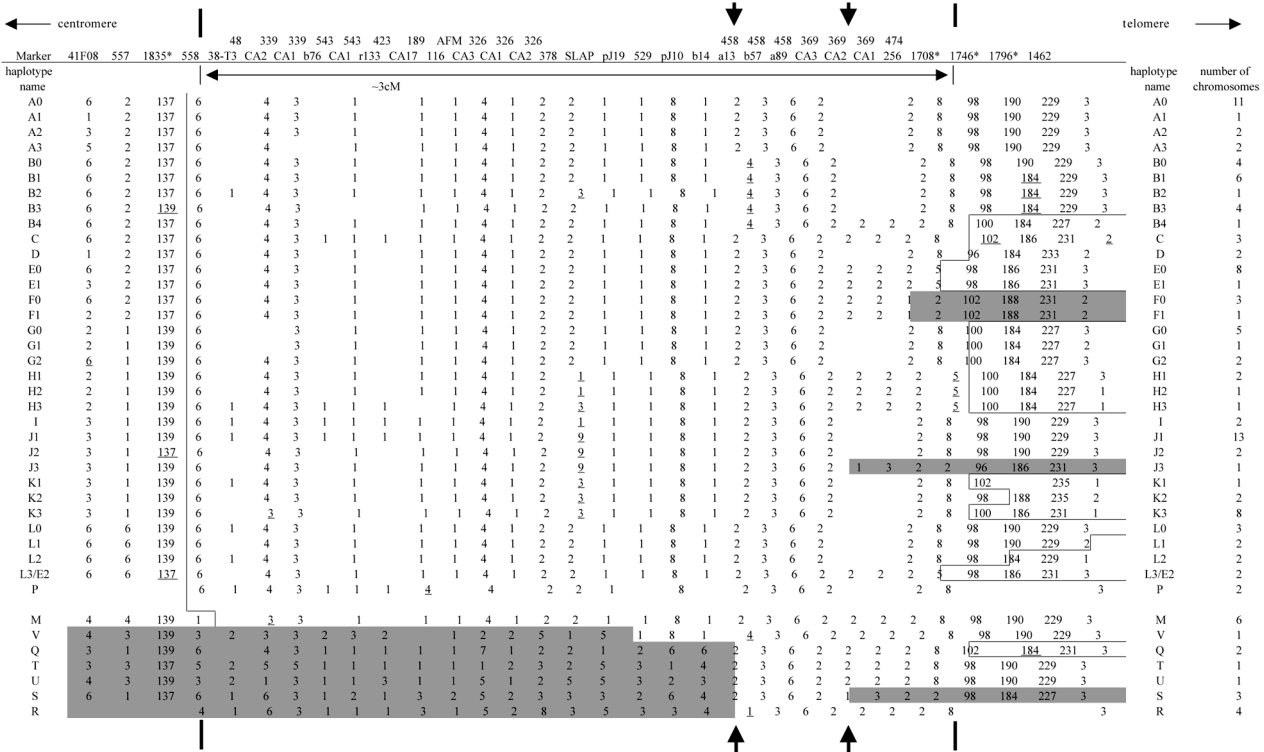
Fig. 1. HMSNL haplotypes. ▨ Borders of initial HMSNL region; ▩ recombinants used in refined mapping; 3, marker mutation; ↑ HMSNL region defined by initial study; ↑ HMSNL region defined by present study; * alleles given as size in base pairs; spaces in the body of the table represent untyped markers.

haplotype name	41F08	557	1835*	558	38-T3	CA2	CA1	b76	CA1	r133	CA17	116	CA3	CA1	CA2	378	SLAP	pJ19	529	pJ10	b14	a13	b57	a89	CA3	CA2	CA1	256	1708*	1746*	1796*	1462	haplotype name	number of chromosomes
A0	6	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	190	229	3	A0	11
A1	1	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	190	229	3	A1	1
A2	3	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	190	229	3	A2	2
A3	5	2	137	6		4				1		1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	190	229	3	A3	2
B0	6	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	4	3	6	2				2	8	98	190	229	3	B0	4
B1	6	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	4	3	6	2				2	8	98	184	229	3	B1	6
B2	6	2	137	6	1	4	3			1	1	1	4	1	2	3	1	1	8	1	4	3	6	2				2	8	98	184	229	3	B2	1
B3	6	2	139	6		4	3			1	1	1	4	1	2	2	1	1	8	1	4	3	6	2				2	8	98	184	229	3	B3	4
B4	6	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	4	3	6	2	2	2	2	8		100	184	227	2	B4	1
C	6	2	137	6		4	3	1	1	1	1	1	4	1	2	2	1	1	8	1	2	3	6	2	2	2	2	8	102	186	231	2	C	3
D	1	2	137	6		4	3	1	1	1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	96	184	233	2	D	2
E0	6	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	5	98	186	231	3	E0	8
E1	3	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2	2	2	2	5		98	186	231	3	E1	1
F0	6	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2	2	2	1	2	102	188	231	2	F0	3
F1	2	2	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2	2	2	1	2	102	188	231	2	F1	1
G0	2	1	139	6			3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	100	184	227	3	G0	5
G1	2	1	139	6			3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	100	184	227	2	G1	1
G2	6	1	139	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	100	184	227	3	G2	2
H1	2	1	139	6		4	3			1	1	4	1	2	1	1	1	8	1	2	3	6	2	2	2	2	5		100	184	227	3	H1	2
H2	2	1	139	6		4	3			1	1	4	1	2	1	1	1	8	1	2	3	6	2	2	2	2	5		100	184	227	1	H2	1
H3	2	1	139	6	1	4	3	1		1	4	1	2	3	1	1	8	1	2	3	6	2	2	2	2	5		100	184	227	1	H3	1
I	3	1	139	6	1	4	3	1	1	1	1	4	1	2	1	1	1	8	1	2	3	6	2				2	8	98	190	229	3	I	2
J1	3	1	139	6	1	4	3	1	1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	190	229	3	J1	13
J2	3	1	137	6		4	3			1	1	4	1	2	9	1	1	8	1	2	3	6	2				2	8	98	190	229	3	J2	2
J3	3	1	139	6		4	3			1	1	4	1	2	9	1	1	8	1	2	3	6	2	1	3	2	2	96	186	231	3	J3	1
K1	3	1	139	6	1	4	3			1	1	4	1	2	3	1	1	8	1	2	3	6	2				2	8	102		235	1	K1	1
K2	3	1	139	6		4	3			1	1	4	1	2	3	1	1	8	1	2	3	6	2				2	8	98	188	235	2	K2	2
K3	3	1	139	6		3	3		1	1	4	1	2	3	1	1	8	1	2	3	6	2				2	8	100	186	231	1	K3	8
L0	6	6	139	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	190	229	3	L0	3
L1	6	6	139	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	190	229	2	L1	2
L2	6	6	139	6	1	4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2				2	8	98	184	229	1	L2	2
L3/E2	6	6	137	6		4	3			1	1	1	4	1	2	2	1	1	8	1	2	3	6	2	2	2	2	5		98	186	231	3	L3/E2	2
P				6	1	4	3	1	1	1	4		4		2	2	1		8		2	3	6	2				2	8				3	P	2
M	4	4	139	1		3	3		1	1	4	1	2	2	1	1	8	1	2	3	6	2	2	2	2	8	98	190	229	3	M	6
V	4	3	139	3	2	3	3	2		1	2	2	5	1	5	4	3	6	2	2	2	8	98	190	229	3	V	1
Q	3	1	139	6		4	3	1	1	1	1	7	1	2	1	2	6	6	3	6	2	2	2	8	102	184	231	3	Q	2
T	3	3	137	5	2	5	5	1	1	1	1	1	2	3	2	5	3	1	4	3	6	2	2	2	8	98	190	229	3	T	1
U	4	3	139	3	2	1	3	1	3	1	5	1	2	5	5	2	3	3	6	2	2	2	8	98	190	229	3	U	1
S	6	1	137	6	1	6	3	1	2	1	3	2	5	2	3	3	2	6	4	3	6	2	1	3	2	2	98	184	227	3	S	3
R				4	1	6	3	1	1	1	3	1	5	2	8	3	5	3	3	4	1	3	6	2	2	2	2	8				3	R	4

was ensured through the use of allelic ladders including representative samples from all gels.

2.2.4. Haplotype analysis and recombination mapping

The order of markers (Fig. 1) was obtained by STS content mapping of the genomic BAC and PAC clones forming the contig. The haplotypes on normal and HMSNL chromosomes were constructed manually in each family and examined for recent and historical recombinations.

3. Results

3.1. The HMSNL phenotype

The participating centres have reported consistent clinical findings which allow the definition of the HMSNL phenotype [1,3,4,6,7]. Onset of symptoms is in the first decade of life. Gait disturbances begin at age 5–10 years and difficulty in using the hands becomes evident at age 5–15 years. Muscle wasting and weakness are distally accentuated and particularly severe in the lower limbs. Tendon reflexes are absent in the lower limbs and, depending on the age of the patient, depressed or absent in the upper limbs. Sensory loss involving all modalities is also distally accentuated and most evident in the lower limbs. Skeletal deformities, parti-

cularly of the feet, are common. Nerve conduction studies show a severe reduction in motor nerve conduction velocity. Sensory potentials are usually unobtainable.

Hearing loss is a characteristic feature of HMSNL and usually develops in the second or third decade. Abnormal BAEP recordings, suggesting retrocochlear involvement of the central auditory pathways, are present in all patients prior to the onset of subjective hearing loss. The deafness in HMSNL has been shown to be of a pure neural type, due to a neuropathy of the auditory nerve with function in the cochlear outer hair cells being preserved [6].

The neurophysiological findings suggestive of a demyelinating neuropathy are supported by the neuropathological observations in peripheral nerve biopsies. An excess of nerve fibres with inappropriately thin myelin sheaths is a consistent finding [2–7]. A number of ultrastructural observations clearly distinguish HMSNL from other demyelinating neuropathies. These include (1) early and severe axonal loss; (2) curvilinear axonal inclusions resembling the dying-back type of distal axonopathy seen in experimental vitamin E deficiency; (3) failure of compaction of the innermost myelin lamellae; (4) a poor hypertrophic reaction of the Schwann cell with the formation of atypical onion bulbs in young patients which regress later in the course of the disease; and (5) pleomorphic inclusions in the adaxonal Schwann cell cytoplasm. The findings point to a possible

Table 1
PCR primer sequences of newly identified markers in the HMSNL region

Marker	Primer sequence ($5' \rightarrow 3'$)		Size of PCR product (bp)	Number of alleles	Repeat motif
	Forward	Reverse			
48 38-T3	AAACAAGTCATCTTAGACA	CACAGCAAGACTCCTTC	98	2	CCTT
339CA2	CGGACCCAAATCAATTTTC	CCATTTACAGTGCAGATG	122	6	CA
339CA1	TTGATCTGGGAGAATGATG	ACATATACACTGCCACG	100	5	CA
543B76	GTGGCAGAGTGAGACACT	TATACTATGACCATTCTCTG	120	2	CA
543CA1	GTCTTACTGCTGTATCTCC	CCACAATACGAATGTATG	150	3	CA
423R133	CATTACAGGCATCTGCCATG	GTGAACATGGCGAACGCTG	120	3	CA
189CA17	GAAAAGGTCAATATGCCAGG	GATTGAGTTGTCTATTTGTC	140	3	CA
326CA3	TCATGGGATAAAACATTAGTGAA	GATTTGCAATTTATTCAAGAACAC	160	7	CA
326CA1	GAAATGCTGGCAGAAGTCTTGAAAG	TTGACTCCCTGCATTTATACCAATCTT	190	2	CA
326CA2	GTGCACCAAAATCTCACAAATCAC	CCAATTCACCGCAAGTCAGACACT	300	7	CA
SLAP	TGCGTCAGAAGACTGTGGAC	TGGCCATGGTTTTCATGTGC	170	6	CA
pJ 19	ACCACAGCCCAGTGCCTGATTCC	TTTACTTGGCACCCAGGCTTCTCA	140	5	CTG
pJ 10	AGGGTCTTAGTCCCAACA	AGAAAGAACTGACCAGCC	170	7	CA
458B14	CTCTCCCTCCAAAGTCTCC	AAAGCAGAGGAAGCGCTGG	170	5	CA
458A13	AAGTATCCCTGTTATTCAGC	CTTACTTCCAGGATAAACAC	110	5	CA
458B57	AGACAGTCTTCTTGACTGG	TGTACCCAAGTCCCATCC	120	6	CA
369A89	CTCATCTACACACTCGCGCG	GGCCGATGAGACGGTCCGAAA	200	11	CA
369CA3	GATATAATTATGCAGATAGG	GTTATTTGTCTTATCAGTC	188	4	CA
369CA2	CTCCTACCTGCTGTCTGC	GCTGAGAAGTCCATGATC	199	3	CA
474CA1	TCAGGCAGGCTGGATTCAG	AGCAGAGCCATGGCACATG	170	4	CA

disruption of Schwann cell–axonal interaction and suggest that the HMSNL gene product may be a growth factor, a growth factor receptor or a signalling molecule involved in the maintenance of the differentiated state of these cells.

3.2. A dense genetic map of the HMSNL region

The initial gene mapping study [1] placed the HMSNL gene between markers D8S558 and D8S256. According to public databases, the interval contained five known polymorphic microsatellites positioned in the order cen-D8S558-D8S529/D8S378-AFM116yh8-D8S256-tel. The construction of the contig and of the genetic map followed this order, starting from the region around D8S529 for which all individuals in the original study had been found to share the same marker allele. In the D8S529-D8S256 interval, we have identified 20 new polymorphic microsatellites.

With two exceptions, markers 4838-T3 and pJ19, all newly identified microsatellites are simple CA repeats (Table 1). pJ19 has a CTG sequence motif but our study has excluded an expansion of this triplet repeat as the cause of HMSNL and has shown pJ19 to be a neutral variant. The markers were found to vary widely in terms of polymorphism. Some, such as 326CA1, 543b76 and 4838-T3, displayed two alleles in the population studied and were therefore of limited value. On the other hand, a number of the new microsatellites of which those located within the critical region are of particular importance, are highly polymorphic and informative, e.g. 369a89 which presented with 11 alleles. These newly identified markers, together with

those previously published, provided a dense genetic map of the HMSNL interval on 8q24.3 with an average intermarker distance of less than 0.1 cM.

Subsequent results from the physical mapping of the region have reversed the original sequence of genomic clones and polymorphisms and placed the known markers in the order cen-D8S558-AFM116yh8-D8S378-D8S529-D8S256-tel (Fig. 1).

3.3. Refined mapping of the HMSNL gene

The 32 markers spanning the entire region were genotyped in the group of 174 individuals, including 60 affected subjects.

The analysis of polymorphic haplotypes (Fig. 1) revealed a number of historical recombinations involving the markers flanking the HMSNL region, namely the centromeric group 41F08, D8S557, D8S1835 and D8S558, and the telomeric group D8S256, D8S1708, D8S1746, D8S1796 and D8S1462.

In the telomeric part of the region, haplotype diversity has been generated by historical recombinations occurring mainly around microsatellite D8S256. For the purposes of refining the HMSNL interval, breakpoints centromeric of D8S256 are of particular interest. Two such historical recombinations had been found in the initial study of the Lom family (Fig. 1, haplotypes E0, E1, L3 and E2, F0 and F1) and two more were identified in the current collection of affected families (Fig. 1, haplotypes J3 and S). The analysis of newly identified microsatellites centromeric to D8S256 has shown that in one of these haplotypes (E0, E1, L3/E2),

the crossover is between D8S256 and the newly identified marker 474CA1 (Fig. 1). The breakpoints in the other three historical recombinations are more proximal and useful for the refined mapping of the gene localisation. The recombination has occurred between 474CA1 and the next proximal marker 369CA2 in one haplotype (Fo, F1). In the remaining two haplotypes (J3, S), the breakpoints map to the interval 369a89-369CA3, thus placing the telomeric boundary of the HMSNL interval centromeric of marker 369CA3.

In the centromeric group of flanking markers, a number of independent recombination events has generated a diversity of haplotypes. In most cases, the breakpoints of these historical recombinations map between D8S1835 and D8S558 and are therefore of no relevance to narrowing down the previously defined HMSNL region. The historical recombination involving marker D8S558 (Fig. 1, haplotype M), identified during the initial analysis [1], was shown in this study to be confined to the original Lom family.

Within the interval D8S558-369CA3, nearly identical haplotypes were shared by the vast majority of disease chromosomes. The only variation observed was at markers D8S378, 189CA17, 339CA2 and 458b14 which presented with several alleles (Fig. 1). The fact that these four markers are flanked by conserved haplotypes suggests that the observed differences are the product of microsatellite mutations rather than historical recombinations. In most cases, the variant allele occurred on a specific haplotype background, suggesting secondary founder effect. With the exception of this variation, the markers in the interval formed a highly conserved haplotype observed in 90% of the HMSNL chromosomes (108 out of 120 analyzed). This haplotype has not been found on any of the 88 normal chromosomes tested.

Unusual haplotypes (Fig. 1, haplotypes Q to V) were found in 12 HMSNL chromosomes from six different families that originate from divergent Romani groups. One of these was obviously the product of a maternal recombination. In four families, the recombinant haplotype was inherited by all affected members, pointing to historical recombinations. Tracing the recombination was not possible in one case, where only an affected individual and no other family members were available for analysis.

In the latter case (Fig. 1, haplotype V) unusual alleles were found for all markers centromeric to pJ19, suggesting that the recombination occurred between markers SLAP and pJ19.

The only recent recombination was observed in individual R5 from an affected family from Bulgaria (Fig. 2). R5 has inherited the normal maternal chromosome for the entire interval centromeric to pJ10. Similar to the other affected members of the family, he is homozygous for the common disease haplotype in the telomeric part of the region.

In family G (Fig. 3), the paternal disease chromosome of affected individual G12 carries the usual HMSNL haplotype (A_0), whereas the maternal chromosome (haplotype T) is

different for the entire region centromeric to pJ10; G12 is also homozygous for the predominant disease haplotype in the telomeric region (Figs. 1 and 3). In the same large kindred (Fig. 3), individual G19 is closely related to the HMSNL branch of the extended family and has inherited the common disease haplotype (A_0) along the entire HMSNL region. This haplotype has been transmitted to her two daughters, G21 and G22. The father in this nuclear family, G20, originates from the same community. His transmitted chromosome carries the high risk haplotype for the whole interval centromeric to pJ10 (inclusive) and a totally different distal haplotype. The two daughters, G21
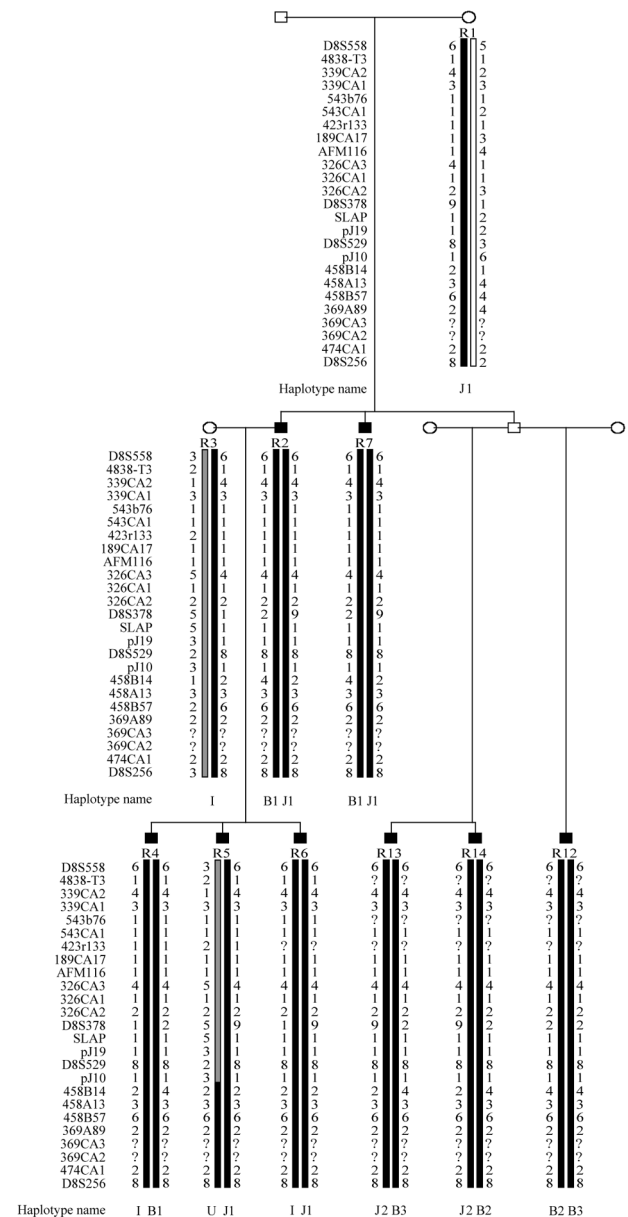

Fig. 2. Pedigree R. Abbreviated haplotypes for HMSNL region showing maternal recombination in R5, with the breakpoint mapping telomeric of pJ10. Haplotype designations as shown in Fig. 1 are noted at the bottom of haplotype bars.

and G22, are not affected by HMSNL. It appears probable that the two recombinant haplotypes segregating in this small endogamous Gypsy group are in fact the product of a single recombination event.

In the Spanish HMSNL family (Figs. 1 and 4), the maternal haplotype (S) inherited by the three affected children is different from the common disease haplotype for the entire region centromeric of pJ10 and also for the telomeric part of the region, distal to 369CA3. All three affected individuals in this family are homozygous for the typical haplotype shared by all HMSNL chromosomes in the interval flanked by pJ10 and 369CA3.

The recombinant haplotype (R) segregating in the Slovenian family [6] was found to occur on the disease chromosomes of two unrelated HMSNL carriers. The recombination in this case clearly involved pJ10 and its proximal markers. The adjacent telomeric marker, 458b14 also presented with an unusual allele (Fig. 1) which was conservatively attributed to a microsatellite mutation, similar to those observed in the non-recombinant haplotypes of the B group (Fig. 1).

Haplotype Q (Fig. 1), with unusual allele sizes for the markers centromeric of pJ10 (inclusive), was identified in two affected siblings referred for analysis from France.

All centromeric recombinant haplotypes observed in this study are shown at the bottom of Fig. 1. The lack of similarity between them indicates that they have been generated by independent crossover events. In most cases the breakpoints map to the same region between markers pJ10 and 458b14, suggesting that it contains a recombination hot spot.

The results of the recombination mapping thus place the HMSNL gene in the interval flanked by newly identified microsatellites pJ10 and 369CA3. Within this interval, a highly conserved four-marker haplotype is shared by all 120 HMSNL chromosomes. Our physical mapping data indicate that the overall distance between these two markers, contained in two overlapping BAC clones, is only 206 kb.

4. Discussion

The investigation of a large number of patients from different countries has demonstrated that hereditary motor and sensory neuropathy type Lom presents with a consistent phenotype allowing the establishment of specific diagnostic criteria. Apart from the early onset and marked reduction of motor nerve conduction velocities, the manifestation that allows the clinical distinction of HMSNL from other autosomal recessive demyelinating peripheral neuropathies is the development of deafness in the second or third decade of life. Abnormal BAEP recording are invariably observed in all affected individuals before the onset of subjective hearing loss and are therefore an important diagnostic tool. The neuropathological features of HMSNL are also

distinctive and point to a characteristic combination of deficient Schwann cell function and early and severe loss of axons. These findings do not allow any conclusions to be drawn concerning the localisation of the primary defect (in the axon or the Schwann cell) and in fact indicate that the genetic defect in HMSNL may affect the complex system of Schwann cell/axon interaction and signalling.
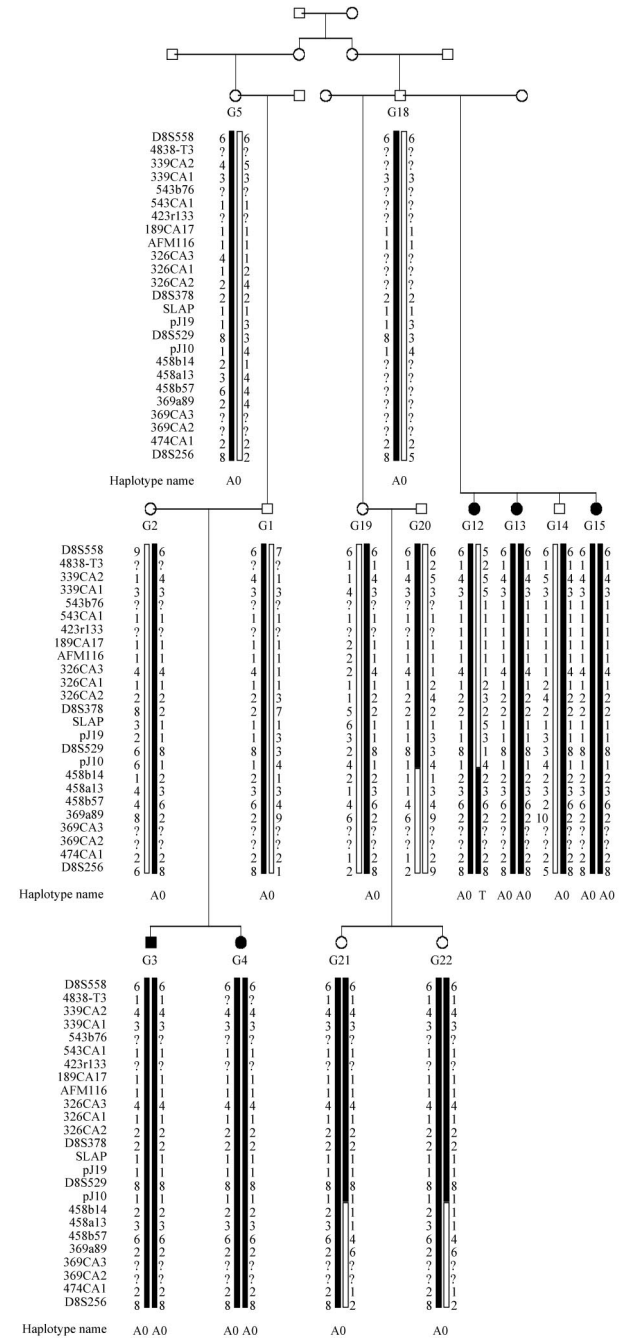

Fig. 3. Pedigree G. Abbreviated haplotypes for HMSNL region showing possibly complementary historical recombinants in G21, G22 (unaffected) and G12 (affected). Haplotype designations as shown in Fig. 1 are noted at the bottom of haplotype bars.
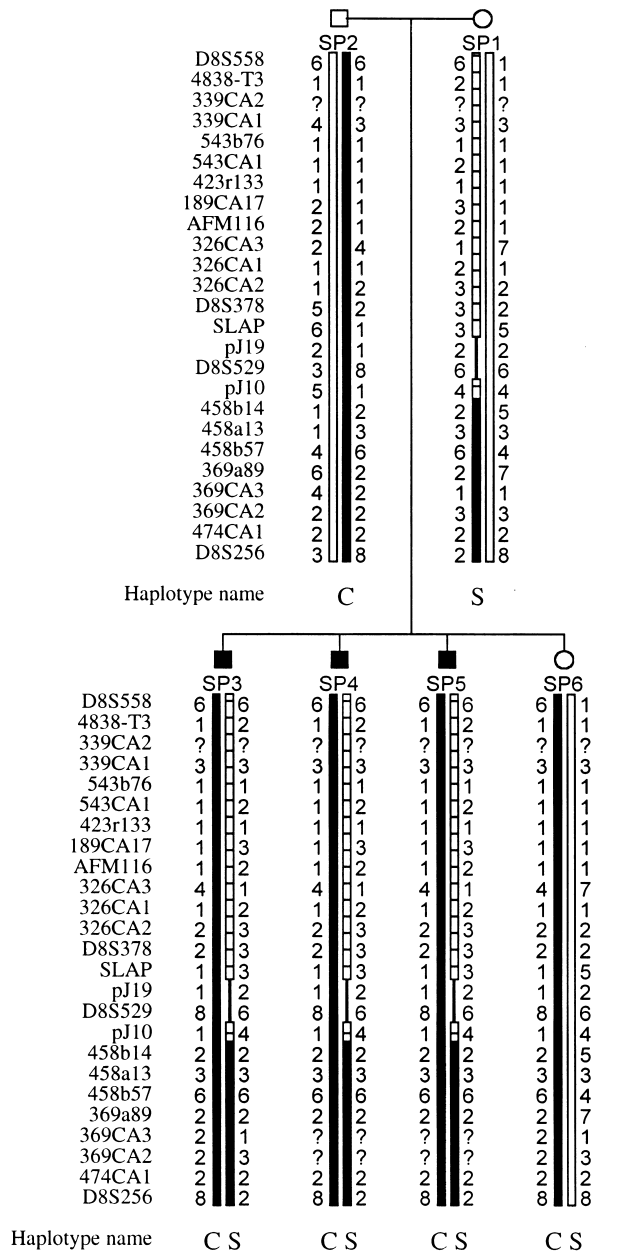
Fig. 4. Pedigree SP (Spanish). Abbreviated haplotypes for HMSNL region showing historical recombination between pJ10 and 458B14. Haplotype designations as shown in Fig. 1 are noted at the bottom of haplotype bars. Uninformative markers centromeric of a breakpoint are indicated by a thin vertical line.

gene interval and reduce its size to a small physical distance of about 200 kb.

Four of the microsatellites identified in this study, namely 458b14-458a13-458b57-369a89, are located within the critical region. Despite the fact that 458b14 displays some variation most likely resulting from marker mutations, the remaining three microsatellites form the highly conserved haplotype 3-6-2 (respectively for 458a13-458b57-369a89) shared by the 120 HMSNL chromosomes and absent in the 88 normal chromosomes tested. While the search for the HMSNL mutation is in progress, these highly informative polymorphisms can be used for carrier testing and prenatal diagnosis based on linkage analysis in known high risk families.

This study also leads to some general conclusions relevant to future research into genetic disorders among the Roma. A number of reports on hereditary neurological disorders among the Roma have been published over the last few years, e.g. limb-girdle muscular dystrophy LGMD2C [9,10], the congenital cataracts facial dysmorphism neuropathy (CCFDN) syndrome [11,12] and congenital myasthenia [13,14] and this population is likely to attract interest in the future as well.

The emerging pattern can be summarised as follows: (a) Gene frequencies are often very high [1,2,10], therefore a rare or novel disorder identified in a single family should be considered a first indicator of a possibly common disease. (b) The Gypsy population of Europe consists of a large number of dispersed small communities which have split from a common ancestral population. The subsequent diversification of polymorphic haplotypes around an old disease mutation, as shown in this study, can be a powerful tool in the refined genetic mapping. International collaborative efforts would be the most productive approach to the identification and study of affected families in this interesting founder population which has no geographical boundaries.

Acknowledgements

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The observed clinical homogeneity is mirrored in the genetic findings. Both the shared region of homozygosity and the closely related flanking haplotypes unambiguously point to a single founder mutation responsible for the disorder in affected individuals across Europe.

This collaborative study of affected families across Europe identified a number of historical recombinations confined to individual divergent Gypsy groups. The information derived from these independent recombination events has allowed us to define precisely the HMSNL

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