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Spinal muscular atrophy in Gypsies from Bulgaria and Hungary

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Spinal Muscular Atrophy is one of the most common autosomal-recessive disorders among Caucasians. It is caused by mutations in the telomeric survival motor neuron (SMN1) gene. We performed a genotype-phenotype correlation study in affected Gypsy subjects from Bulgaria and Hungary, where mild and severe spinal muscular atrophy forms were observed. We found three different gene defects, which in different combinations cause disease of varying severity. Our data support the concept that, although not protective against disease development, the SMN2 gene acts as a modifier in a dose dependant manner. However, the differences in severity observed among patients sharing identical gene defects suggest that additional factors should contribute to the clinical phenotype.

Key words: Spinal muscular atrophy, survival motor neuron, Gypsies.

Introduction

Spinal Muscular Atrophy (SMA) is the most common form of motor neuron disease in children and young adults. According to the age of onset and achieved motor functions, the disease is classified into four major forms (1). The clinical signs of the most severe SMA type (SMA I, Werdnig-Hoffmann disease) are evident in utero or soon after birth. The affected infants have profound hypotonia and generalized weakness and are never able to sit unaided. The intermediate type SMA (SMA II) has an onset within the first year of life. The children are normal at birth, they can sit, but never achieve the ability to stand or walk independently. In type III (Kugelberg-Wellander disease), there is a broader range of age of onset, from the first year of life until the third decade (2). Patients with that type can stand and walk. The mildest form of proximal SMA (SMA IV) has an onset in adulthood and ambulation is often maintained for decades after the first signs of weakness.

The disease is caused by mutations in the SMN1 gene (3). This gene lies in a large inverted duplication together with three other genes – NAIP, BTF2p44 and H4F5 (3-6). Due to the duplication of the region, all genes are present in two copies. The SMN gene has a centromeric (SMN2, partially active) and a telomeric (SMN1, fully active, involved in causing the disease) copies, showing >99% homology.

There are two major types of molecular defects in SMA: true gene deletions and gene conversions. Deletions eliminate the whole SMN1 gene, alone or together with the neighbouring genes (7, 8), and are usually found in patients with the most severe SMA phenotype (8-11). In gene conversions, mainly implicated in the milder forms of the disease, part of SMN2 is copied into SMN1, whereby the donor gene remains unaffected whereas the acceptor becomes a hybrid SMN1/2 gene (7). The prevalent genotype/phenotype model predicts that type I SMA patients carry two "severe" alleles with gene deletions, type II patients are compound heterozygotes for a "mild"

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and a "severe" SMA allele, while type III patients have two "mild" type alleles generated by gene conversions (9, 10). A number of reports on significant intrafamilial clinical variation suggest that other factors, in addition to the SMN1 gene, may play a role in phenotype determination (12-14). SMA is a common disorder occurring in all ethnic groups. The combined incidence of the different forms is approximately 1 in 6,000-10,000 live births (15). However, these are significant deviations from there figures, especially in isolated populations such as the Egyptian Karaites (16) and Reunion Islands Europeans (17). No information on the molecular basis of SMA in these two or on any other isolated populations has been reported.

Here we present data on the molecular and phenotypic characteristics of SMA in affected Gypsy subjects from Bulgaria and Hungary.

Material and Methods

The study included a total of 38 families - 30 from Bulgaria and 8 from Hungary. Diagnosis was based on the clinical criteria proposed by the International SMA Consortium (18). The overall number of individuals participating in the genetic study was 147, of whom 45 were affected and 102 unaffected. The analysis of the clinical course of the disease was performed on an extended group of 56 individuals including the 45 patients mentioned above plus 11 deceased affected siblings, assuming identical genotypes within sibship. A total of 78 unrelated chromosomes were investigated. The analysis included screening for deletions of exons 7 and 8 of the SMN genes and exon 5 of the NAIP gene, and genotyping for five microsatellite markers in the SMA region (D5S681, D5S435, D5S610, C212, C272). The analytical protocols were as described (4, 19, 20). Marker haplotypes were constructed manually, following the order *cen-D5S681 - D5S435 - C212 - C272 - D5S610 - tel.* Haplotype analysis included only one affected individual per sibship.

Results

Genetic analysis

The majority (66 out of 78) Gypsy SMA chromosomes presented with three haplotypes, referred to as A, B and C (Fig. 1). These three haplotypes were related and shared conserved

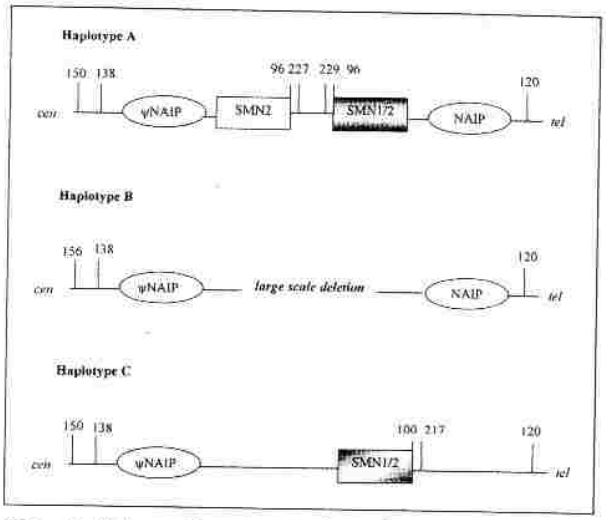

Fig. 1. Schematic presentation of three main haplotypes observed among the Gypsy SMA patients. Alleles of the analyzed markers are given in base pairs, following the order: cen-D5S681-D5S435-C212-C272-SMA gene region-D5S610-tel.

marker alleles, pointing to a common origin of the SMA chromosomes in this endogamous population (Jordanova et al., Neuromuscular Disorders, in press).

Deletion analysis demonstrated different gene defects carried by the three related Gypsy SMA haplotypes. Chromosomes with haplotype A were shown to have one SMN2 copy and one hybrid SMN1/2 gene. Haplotype B was characterised by a large-scale deletion, involving both SMN copies and leaving the NAIP gene intact. Haplotype C chromosomes presented with a single hybrid SMN1/2 gene and no SMN2 or NAIP genes. The patients included in the study could be classified into 5 genotype groups, which did not represent all possible haplotype combinations. The observed genotypes included A/A, A/B, A/C, C/C, and Others (Table 1). No B/B homozygotes or B/C compound heterozygotes were identified.

Clinical findings

Patients with genotype A/A These individuals are assumed to have two SMN2 copies, two hybrid SMN1/2 genes and two NAIP genes. All were classified as having SMA type III or IV. The age at onset in this group ranged between 2 and 17 years, with difficulties in climbing stairs as a common initial symptom. Loss of ambulance occurred late in life and generally was in corre-

Table 1. Clinical data of the Gypsy patients classified according to their genotype (Modified from Jordanova et al., Neuromuscular Disorders, in press).

Genotype group	Underlying genotype (number of SMN and NAIP copies)	Number of patients	SMA type	Phenotype characteristics		
				Onset	Loss of ambulance	Death
A/A	2 SMN1/2 2 SMN2	17	III	2 y-15 y	5 y-36+y	No
		1	IV	17 y	No/43 y	No
	2 NAIP					
A/B	1 SMN1/2 1 SMN2 2 NAIP	18	I	0-4 mo	Yes	1 mo-6 mo
A/C	2 SMN1/2	5	I-II	0-5 mo	Yes	1.5 y-5 y
	1 SMN2	2	II	6 mo-9 mo	Yes	4 y
	1 NAIP	3	II-III	1 y	2 y-3 y	No
C/C	2 SMN1/2	3	I	At birth	Yes	9 mo
Others	Various	4	I	0-3 mo	Yes	4 mo-1 y
		1	II	6 mo	Yes	No
		2	III	1,5 y-2 y	4 y	No

lation with the age at onset of the disease. Spine deformities (scoliosis) were observed in 50% of A/A homozygotes. Despite the generally similar phenotype in this group, evidence of clinical heterogeneity was also present. For example, in a Bulgarian family with three affected sibs (two girls and one boy), the onset in the two female patients was at age of 12 and 14 and loss of ambulation at 31 and 33 years, respectively. The initial complaints in the affected brother occurred at age 17 and the patient is still ambulant at age of 43, with only subtle walking difficulties and well preserved muscle strength of the upper limbs.

Patients with genotype A/B These patients have one copy each of SMN1/2 and the SMN2 genes (both contributed by haplotype A) and two intact NAIP genes. All were unequivocally classified as having SMA type I. The onset was *in utero*, with diminished foetal movements, or within the first month of life. Low weight and marked hypotrophy at birth were characteristic features. In six patients, chest deformities have been found at first examination in the neonatal period. Death usually occurred during the first six months of life.

Patients with genotype A/C Compound heterozygotes for haplotypes A and C carry two SMN1/2 hybrid genes, and one SMN2 and one NAIP gene, both contributed by the haplotype A chromosome. The 10 patients in this group displayed substantial variability in the course of the disease, classified as types I, II and III. Five patients were diagnosed as intermediate type I-II, with onset of symptoms soon after birth (similar to type SMA I), but prolonged survival to 1.5-5 years of age. They were never able to sit without support, to stand or walk. Two affected siblings, related to a family where SMA type I-II was observed, were diagnosed as having SMA type II. They achieved the ability to sit alone, but were never able to walk. The remaining three patients with that genotype had a milder phenotype, consistent with SMA type III, however loss of ambulance occurred at age 2-3 years. In general, this group was characterised by rapid progression of the disease towards loss of ambulance, severe weakness of the lower and upper limbs and skeletal deformities.

Patients with genotype C/C Homozygosity for haplotype C was found in one Bulgarian patient and was presumed to exist in his two deceased brothers (Fig. 2). Haplotype C homozygotes carry two hybrid SMN1/2 genes, with no preserved SMN2 or NAIP genes. The three affected siblings, all classified as SMA type I, had symptoms of the disease in the newborn period and death occurred at age 9 months.

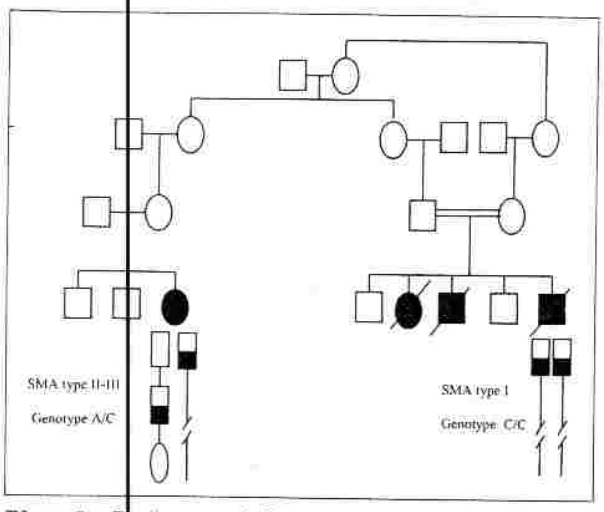

Fig. 2. Pedigree of Bulgarian family with related patients having different genotype combinations and different SMA type.

Discussion

The Gypsy population is a unique isolate spread across the entire European continent, where a number of private genetic disorders have been described and characterized in recent years (21). A common feature of all monogenic "Gypsy" disorders known so far is their genetic homogeneity, where affected individuals carry a single founder mutation occurring on closely related haplotype backgrounds. So far, spinal muscular atrophy, which is the second most frequent autosomal-recessive disorder in Caucasians after cystic fibrosis, has not been considered specific to Gypsy population. Our findings indicate that SMA does occur in some Gypsy groups, however the molecular findings differ from those reported for other disorders. While related conserved haplotypes are observed in most Gypsy SMA patients, they carry different mutations and thus present a unique opportunity for the study of genotype-phenotype correlations. In general, there was a good correlation between the number of preserved SMN copies in the region and the resulting phenotype. A/A homozygotes, who carried the highest number of SMN2 genes, were invariably classified as having SMA type III or IV. It is important to note however, that while homozygosity for haplotype A was found to result in SMA type III, a single A allele in combination with any of the other haplotypes was always associated with SMA type I. The most severe phenotype was observed in C/C homozygotes and in compound A/B heterozygotes, i.e. in the groups of patients where the underlying genotypes were either two SMN1/2 hybrid copies, or a combination of one SMN2 copy, one hybrid gene and a large scale deletion. We have failed to detect any patients with the B/B and B/C haplotype combinations, which can be predicted to result in an extremely severe form of SMA (possibly the SMA type 0, proposed by Dubowitz, (22)). Our findings are supported by the experimental data of Monani et al., who demonstrated early embryonic lethality prior to implantation in homozygous null mice (23). An intermediate and variable phenotype was observed among A/C heterozygotes. The presence of inter- and intra-familial variation is most evident when comparing patients' ability to sit and walk. Five of the children were never able to sit without support, stand or walk. Two patients could sit unsupported, but never started walking. The remaining three patients developed normally up to the age of one year and started walking, but lost their ambulation in two-three years. We observed a Bulgarian family with four affected siblings with different motor abilities. Two displayed marked hypotonia at birth, difficulties in head lifting, turning to the side, and were never able to sit unsupported. The other two siblings manifested their first symptoms at age 6-9 months. They could sit without support but never started walking. Taken together, our data support the concept that, although not protective against disease development, the SMN2 gene acts as a modifier of the disease phenotype. Our findings suggest that the modifying effect of SMN2 on the SMA phenotype is exerted in a dose-dependant manner. However, given the intra-familial phenotype variation observed in this study and others (12-14), the SMN2 copy number appears to be the major among a number of genetic and epigenetic modifiers of the disease phenotype. Additional genetic polymorphisms, non-allelic mutations, and environmental influences should be expected to contribute to the differences between individuals (24).

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