Clinical Study

Clinical and Genetic Study of Algerian Patients with Spinal Muscular Atrophy

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Received 20 December 2012; Revised 12 February 2013; Accepted 18 February 2013

Academic Editor: Haluk Topaloglu

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Spinal muscular atrophy (SMA) is the second most common lethal autosomal recessive disorder. It is divided into the acute Werdnig-Hoffmann disease (type I), the intermediate form (type II), the Kugelberg-Welander disease (type III), and the adult form (type IV). The gene involved in all four forms of SMA, the so-called survival motor neuron (SMN) gene, is duplicated, with a telomeric (tel SMN or SMN1) and a centromeric copy (cent SMN or SMN2). SMN1 is homozygously deleted in over 95% of SMA patients. Another candidate gene in SMA is the neuronal apoptosis inhibitory protein (NAIP) gene; it shows homozygous deletions in 45–67% of type I and 20–42% of type II/type III patients. Here we studied the SMN and NAIP genes in 92 Algerian SMA patients (20 type I, 16 type II, 53 type III, and 3 type IV) from 57 unrelated families, using a semiquantitative PCR approach. Homozygous deletions of SMN1 exons 7 and/or 8 were found in 75% of the families. Deletions of exon 4 and/or 5 of the NAIP gene were found in around 25%. Conversely, the quantitative analysis of SMN2 copies showed a significant correlation between SMN2 copy number and the type of SMA.

1. Introduction

Spinal muscular atrophies (SMAs) are a group of motor neuron disorders characterized by degeneration of spinal cord anterior horn cells, leading to muscular wasting and atrophy [1]. SMA is the most common autosomal recessive disorder after cystic fibrosis, with an estimated 1/10,000 incidence and a 1/60 carrier frequency [2]. Affected patients are classified into four groups according to age at onset and phenotype severity [3, 4]. Type I SMA or the Werdnig-Hoffmann disease (OMIM No. 253300) is the most severe form, with an onset within the first 6 months of age, severe generalized muscle weakness with hypotonia, and death before two years of age. In type II SMA (OMIM No. 253550), affected children sit unassisted, may be able to walk for a short distance, and usually survive over 10 years of age. Type III SMA or the Kugelberg-Welander disease (OMIM No. 253400) has its onset in the first to third decade. Though its course is highly variable, patients are constantly able to walk unassisted. Type IV SMA or adult-onset SMA (OMIM No. 271150) is quite rare.

The survival motor neuron (SMN) gene, implicated in the four forms of SMA, maps to chromosome 5q 11.2–13.3 [5–7] and is duplicated as telomeric and centromeric copies, so called SMN1 (OMIM No. 600354) and SMN2 (OMIM No. 601627), respectively [8, 9]. SMN1 and SMN2 comprising 8 exons are highly homologous, with only five base-pair differences within their 3′ ends [8, 10], and thus encode nearly identical proteins. Two of these base-pairs, located in exons 7 and 8, allow SMN1 to be distinguished from SMN2 at DNA and RNA levels and are currently used for detection of SMN1 deletions [11]. A vast majority (90–98%) of SMA patients have homozygous deletions of SMN1 exons 7 and 8 [8, 12, 13], the remaining ones carrying SMN1 intragenic
Table 1: Classification of autosomal recessive proximal spinal atrophy as defined by Zerres et al. [26].

| Type | Definition |
|------|------------|
| I    | Never able to sit |
| II   | Able to sit but not to walk |
| III  | Able to walk |
| III (a) | Onset before 3 years |
| III (b) | Onset 3–30 years |
| IV   | Onset after 30 years |

mutations [8, 14, 15], with a frequency higher in type I than in types II and III.

Conversely, SMN2 homozygous inactivation is not directly responsible for SMA [8]. A number of studies have however shown that SMN2 acts as a modulator of SMA severity, with an inverse correlation between the SMN2 copy number and the disease severity [16, 17]. Failure of SMN2 to fully compensate homozygous loss of SMN1 is due to a sequence difference in exon 7 which causes alternative splicing of the SMN2 gene, and subsequently lower amount of full-length protein [8, 18, 19].

The neuronal apoptosis inhibitory protein gene (NAIP) [20], close to the SMN genes (15, 5 kb) at q51–q13, was initially considered as a candidate gene for SMA [12, 20, 21]. While subsequent studies have ruled out its direct responsibility, for this disease [17, 22], NAIP has however shown to be more frequently mutated in SMA affected patients than in general population, with homozygous deletions in 45–67% of type I and 20–42% of type II/type III SMA patients [12, 20, 23–25].

Analysis of deletions encompassing both NAIP and SMN genes in a large number of SMA patients suggests that loss of NAIP may be associated with a higher disease severity [10, 20]. Here we investigated the clinical and molecular characteristics of 92 Algerian SMA patients from 57 families to assess the prevalence of SMN1 deletions and the combined impact of SMN2 copy number and NAIP deletions on clinical severity.

2. Materials and Methods

2.1. Patients. 92 patients from 57 Algerian families were diagnosed as having SMA on the basis of clinical findings and electromyoneurography. All patients fulfilled the diagnostic criteria for proximal SMA, as defined by the International SMA Collaboration [26] and by Zerres et al. [27] (Table 1). Inclusion and exclusion criteria were similar to those proposed by the International SMA Collaboration [26]. Patients with symmetrical, muscle weakness of trunk and limbs, proximal muscles weakness more than distal, lower limbs involvement more than upper limbs, and fasciculations of tongue and tremor of hands and in whom denervation was seen on EMG were included in our study. Patients who presented with CNS dysfunction, sensory loss, eye or facial muscle weakness, or involvement of other organs were excluded from this study. A complete clinical history was recorded with emphasis on age, sex, age at onset, course of the disease, perinatal history, parental consanguinity, and affected relatives. Clinical examination focused on neurological parameters, tone, power, reflexes, wasting and atrophy of muscles, and abnormal movements and sensations. Other investigations included serum creatine phosphokinase (CPK), electromyogram (EMG), and nerve conduction velocity.

2.2. Methods. After informed consent, DNA was extracted from peripheral blood samples according to a standard technique [28].

2.2.1. Molecular Analysis of SMN Genes. Search for SMN1 exons 7 and 8 deletions was performed by PCR and restriction enzyme digestion, as described in [29]. The SMN2 copy number was determined by Multiplex Ligation-dependent Probe Assay [30, 31].

2.2.2. Molecular Analysis of the NAIP Gene. All individuals were also tested for exons 4 and 5 deletion of the NAIP gene. PCR conditions and primers used to amplify exons 4 and 5 were identical to those of Roy et al. [20].

3. Results

3.1. Clinical and Genealogical Findings. The rate of consanguineous marriage in this study was approximately 47%. Degrees of consanguinity are listed in Table 2.

Twenty-two of the 57 families (39%) were multiplex. The most common type in our cohort was type III, with fifty-three (53) affected cases from 31 families (60%), followed by type I with 20 cases from 14 families (25%). Frequency of the different types is summarized in Table 3.

In the SMA type I group, the age at onset varied from birth to 6 months, with an average of 5 ± 2, 5 months. All patients were mentally alert. The main symptom was severe hypotonia with poor limb mobility. Only two patients could
Table 4: Phenotypic and genotypic analysis of the 6 SMA type I index patients with prolonged survival.

| Phenotypic and genotypic analysis | N° 1 | N° 2 | N° 3 | N° 4 | N° 5 | N° 6 |
|----------------------------------|------|------|------|------|------|------|
| Sex                              | F    | M    | M    | M    | M    | M    |
| Consanguinity                    |      |      |      |      |      |      |
| Age at onset (months)            | 4    | 2    | 4    | 6    | 4    | Birth |
| Age at diagnosis (year)          | 4    | 5    | 5    | 3    | 2    | 2    |
| Age at last information (months) | 50   | 60   | 72   | 39   | 48   | 41   |
| Lifting of head                  | +    | +    | +    | +    | +    | -    |
| Hypotonia and muscle weakness    |      |      |      |      |      |      |
| Deep tendon reflexes             |      |      |      |      |      |      |
| Fasciculations                   |      |      |      |      |      |      |
| Bulbar symptoms                  |      |      |      |      |      |      |
| Breathing difficulties           |      |      |      |      |      |      |
| Frequent pneumonia               | +    | +    | +    | +    | +    | +    |
| Age at death (months)            | 54   | -    | -    | -    | -    | -    |
| SMN1 gene deletion               | Yes  | Yes  | Yes  | No   | Yes  | Yes  |
| NAIP gene deletion               | Yes  | No   | No   | No   | No   | No   |
| SMN2 copy number                 | 2    | 2    | 3    | 3    | 3    | 3    |

hold up their heads, for a short period of time. Twelve patients (60%) died between 2 and 53 months of age, due to respiratory failure following respiratory tract infections. The remaining ones are still alive (the oldest patient is currently 72 months old). All patients with prolonged survival suffered from joint contractures caused by progressive muscular atrophy, spine deformation as scoliosis or kyphoscoliosis, and swallowing difficulties. The Bulbar symptoms were observed in 5 patients of them (Table 4). They were not able to cope with everyday routine and thus were totally family dependent. The DNA analysis showed that the SMN1 gene is interrupted in five out of six SMA type I patients with prolonged survival, and only one patient showed homozygous deletion of NAIP gene (Table 4). The SMN2 copy number was determined in 5 SMA type I patients carrying homozygous SMN1 deletions, and two of them (2/5) had two SMN2 copies, the remaining ones (3/6) carrying 3 SMN2 copies (Table 4).

In the SMA type II group, age of onset ranged from 8 to 18 months (average 12, 7 ± 3, and 3 months). They were defined by ability to sit alone. Some children experienced early difficulty for sitting or rolling over (2 patients), while 3 patients were able to crawl and stand with support at a mean age of 22 months for a period of 4 months, and two patients were able to walk with support for a period of 6 months. None walked unaided. Scoliosis and contractures constantly developed in the patients who all became wheelchair dependent (6/16). In the type II group all patients are still alive, and 4/16 patients (25%) survive beyond age 15. In the SMA type III group, clinical onset ranged from the first year of life to the 3rd decade. Twenty-two patients (41, 5%) were confined to a wheelchair at ages ranging from 10 to 34 years, the remaining ones being still able to walk, with support. Hand tremor was found in 26 of 53 patients type III. Distal muscle weakness and/or atrophy was associated with the classical proximal defect, with frequent spine deformities and contractures in 39 patients. Life span was not significantly reduced.

SMA type II and III patients coexisted within 2 families. In the three adult-onset SMA patients (type IV), age of onset ranged from 20 to 41 years (mean age of onset 30 ± 8 years). Adult SMA patients, except for patient 2, had very mild phenotypes, compared with the childhood onset.

Blood CPK activity was normal in all SMA type I patients and was occasionally normal or slightly elevated in patients with type II (3 patients) or type III SMA (14 patients). In EMG examination the increased mean potentials, amplitude, duration, and area were stated. Maximal effort pattern in both proximal and distal muscles was reduced; spontaneous activity fibrillation and occasional fasciculations were present. Motor conduction velocity and sensory nerve conduction were normal.

3.2. Molecular Findings. Homozygous deletions of SMN1 exon 7, exon 8, or both were observed in 43/57 families (75%) with the following distribution: type I 11/14, type II 7/10, type III 24/31, and type IV 1/2. Among the 43 families with deletions, 36 had both exons 7 and 8 deleted, while four had deletions only of exon 7, and 3 patients carried only homozygous deletion restricted to SMN1 exon 8 (Table 5). Homozygous deletions of exons 4 and/or 5 of the NAIP gene were found in 4/14 type I, 2/10 type II, 9/31 type III, and 0/2 type IV families (Table 5). Homozygous NAIP deletions were frequently associated with homozygous SMN1 deletions.

The SMN2 copy number was determined in patients carrying homozygous SMN1 deletions n = 62 (Table 6). II/15 (73%) SMA type I patients had one or two SMN2 copies, the remaining ones carrying 3 SMN2 copies, 10/12 (83%) type II patients carried three or four SMN2 copies, the remaining ones having 2 SMN2 copies, and 32/33 (96%) type III patients
We analyzed three genes implicated in SMA. Discussion

SMN patients carried at least five copies. Finally, both adult onset patients carried at least five SMN2 copies (Table 6).

### 4. Discussion

We analyzed three genes implicated in SMA, namely, SMN1, SMN2, and NAIP, in a cohort of 92 SMA affected patients from 57 Algerian families, in an attempt at phenotype/genotype correlation. All patients fulfilled the diagnostic criteria for proximal SMA, as defined by the International SMA Collaboration [27] and were classified into four subgroups according to the criteria of Zerres et al. [26]. Twenty patients had type I, 16 patients type II, 53 type III, and 3 patients type IV SMA. Although clinical classification of SMA is helpful in providing medical care and prognostic assessment; it is however based on subjective and arbitrary parameters which may still be controversial and subject to errors. Zerres and Rudnik-Schöneborn [32], in a retrospective study of 445 SMA patients, found 106 cases (24%) that could not be classified and suggested subdividing type III SMA into two groups, resulting in a total of four SMA types. In the present study, clinical classification of patients into four groups, based on criteria of the International SMA collaboration [27] and of Zerres and Rudnik-Schöneborn [32], was possible for most patients. In these classifications, age at onset is classically considered to be predictive of the outcome. However, in II cases (12%) age at onset and/or death and motor milestones (ability to walk independently) did clearly overlap between two subsets. It is thus important to keep in mind the possibility of long-standing disease courses with an early onset of weakness compatible with a prolonged survival. For example, 6 patients with SMA type I survived over age two. In 5 patients, age at onset was before 18 months, which is characteristic of type II SMA, while walking capacities were compatible with SMA type III. Coexistence of various types of SMA (II and III) within a given family occurred in our series (2/57 families), as reported elsewhere [33, 34], in favor of a continuous spectrum in childhood SMA. Additionally we found a predominance of males to females (17 female/36 males) in type III SMA, as previously reported by Rudnik-Schöneborn et al. who suggested the presence of a female sparing factor [35]. Tazir and Geronimi reported the same fact in a much larger Algerian series in which chronic cases were predominant [36].

Consanguinity rate was 47% in our cohort, that is above the average reported in the Algerian general population (≈39%) [37]. Furthermore twenty-four families (42%) had a positive history of affected relatives. These data emphasize the importance of lowering the consanguinity rate and the value of genetic counseling and prenatal diagnosis for preventing SMA in our community.

From molecular point of view, the proportion of SMN1 homozygous deletions was 75% in our study, lower than those found in several other previously reported population studies [38–42] (Table 7).

Deletions involving both exons 7 and 8 were observed in 36 families (63%), being much more frequent than deletions restricted to exon 7 (4 families, 7%) or 8 (3 families, 5%), in agreement with previous investigations [8, 23, 42, 43].

Several authors reported a frequency of large deletions, encompassing both SMN and NAIP genes, higher in SMA type I than in the other types [12, 25, 43–46].

In our study the frequency of NAIP gene deletions was 28%, 20%, and 16% for type I, II, and III, respectively, and did apparently not influence the disease severity.

Moreover, a great proportion of severely affected patients harboured no NAIP deletion, and the same pattern of deletions (involving SMN and NAIP genes) was found among affected sibs with different phenotypes (SMA II and SMA III). This supports the hypothesis that other factors may regulate the severity of the clinical course in addition to the extent of the deletion [17, 47]. The SMN2 gene was consistently present as at least one copy in our series, thus contributing to some amount of SMN protein [46, 48]. It has previously been reported that most SMN2 transcripts lack exon 7 and are thus functionally defective, reinforcing the view that the disease is the result of an insufficient amount of intact SMN protein [49]. Interestingly, no patient has been diagnosed

Table 5: Distribution of homozygous deletion of SMN1 and NAIP genes according to the different types of SMA.

| N of families | Exon 7 | Exon 8 | Exons 7 and 8 | NAIP gene deletion |
|--------------|--------|--------|--------------|-------------------|
| SMA I (n = 14) | 2 (14%) | 1 (7%) | 8 (57%) | 4 (28%) |
| SMA II (n = 10) | 1 (10%) | 0 | 6 (60%) | 2 (20%) |
| SMA III (n = 31) | 1 (3%) | 2 (6%) | 21 (67%) | 9 (29%) |
| SMA IV (n = 2) | 0 | 0 | 1 (50%) | 0 |

Table 6: Analysis of the SMN2 copy number in the 62 patients with homozygous absence of the SMN1 gene.

| SMA type | N patients | 1 | 2 | 3 | 4 | 5 |
|----------|------------|---|---|---|---|---|
| I        | 15         | 2 (13%) | 9 (60%) | 4 (26%) | 0 | 0 |
| II       | 12         | 0 | 2 (16%) | 7 (58%) | 3 (25%) | 0 |
| III      | 33         | 0 | 0 | 5 (15%) | 27 (81%) | 1 (3%) |
| IV       | 2          | 0 | 0 | 0 | 0 | 2 (100%) |
Table 7: Frequency of SMN1 homozygous deletion in SMA around the world.

| References | Countries          | N patients | SMN Del (%) |
|------------|--------------------|------------|-------------|
| [38]       | Korea 2001         | 37         | 32.43%      |
| [39]       | Vietnam 2003       | 17         | 41.17%      |
| [40]       | Johannesburg 2007  | 92         | 51%         |
| [41]       | Egypt 2001         | 33         | 55%         |
| [42]       | Russia 2001        | 57         | 65%         |
| [33]       | Brasilia 1999      | 87         | 69%         |
| **Our study** | **Algeria 2009**  | 92         | 75%         |
| [43]       | India 2005         | 45         | 76%         |
| [44]       | Saudi Arabia 1997  | 16         | 82%         |
| [55]       | Morocco 2003       | 54         | 83.33%      |
| [56]       | Turkey 2000        | 60         | 85%         |
| [57]       | Germany 1995       | 195        | 90%         |
| [58]       | Spain 1995         | 54         | 91%         |
| [59]       | Holland 1995       | 103        | 93%         |
| [60]       | Japan 2002         | 32         | 94%         |
| [61]       | Tunis 2006         | 60         | 95%         |
| [62]       | Iran 2004          | 22         | 95.40%      |
| [24]       | UK 1995            | 140        | 97.80%      |
| [63]       | Kuwait 2001        | 46         | 97.82%      |
| [8]        | France 1995        | 229        | 98%         |
| [64]       | Taiwan 1995        | 42         | 100%        |

with a homozygous absence of both SMN1 and SMN2 gene so far, suggesting that a total absence of SMN would be lethal in utero.

The results of our quantitative analysis of SMN2 gene copies clearly show that the disease phenotype is influenced by the number of copies of the SMN2 gene, consistent with previous studies indicating that type II and III patients have on average a larger number of SMN2 copies than type I SMA patients [50–53]. In our series of II SMA type I patients who had a determination of the SMN2 copy number, the two patients with one SMN2 copy had a median survival of 5 months, whereas those with two and three SMN2 copies survived 8 and 23 months, respectively.

It is classically admitted that the SMN2 copy number is less than 3 in SMA type I and at least 3 in SMA type II, III, and IV [52–54]. Such a correlation between the number of SMN2 genes and the clinical phenotype is however not conclusive.

In conclusion, our results are in agreement with the general consensus that there is no correlation between the size of SMN1 deletions and the clinical severity of SMA and that there exists a close relationship between SMN2 copy number and SMA disease severity, suggesting that the determination of SMN2 copy number may be a good predictor of SMA disease type. We suggest that other still unknown factors may regulate the severity of the clinical course and influence phenotype expression. Our study additionally understanding the function of the SMN protein would probably be the key in unraveling the molecular basis of SMA.

Electronic Database Information

Accession numbers and the URL for data presented herein are as follows: Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for type I SMA [MIM 253300], type II SMA [MIM 253550], type III SMA [MIM 253400], SMN1 [MIM 600354], and SMN2 [MIM 601627]).

Acknowledgments

The authors thank the SMA families and clinicians who collaborated with them in this study. They are also grateful to Professor Assia Benhabiles, Dr. Soumeya Lemai, Professor Louis Violet, and Professor Michel Koenig.

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