Descriptive Epidemiology of Spinal Muscular Atrophy Type I in Estonia

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Introduction

Spinal muscular atrophy (SMA) is an autosomal-recessive disorder caused by the degeneration and loss of the α-motor neurons in the anterior horn of the spinal cord [1, 2] resulting in muscular weakness and atrophy. Three clinical types of the disease (type I, II and III) have been recognized, based on the age of onset, clinical severity and life span [2, 3], SMA I being the most severe form. SMA is caused by mutations in the telomeric spinal muscular atrophy gene (SMN1) on chromosome 5q13 [4]. Exons 7 and 8 of the gene are homozygously deleted in 90–95% of patients with childhood-onset SMA [5, 6]. Smaller intragenic mutations of the gene have also been found [4, 7]. The SMN1 gene is 99% identical with its centromeric copy SMN2. The loss of SMN2 alone does not cause SMA and is not considered to be disease causing [4]. However, the number of copies of SMN2 gene plays a role as a phenotypic modifier factor [8, 9].

SMA is the second most frequent autosomal-recessive disorder in Europeans after cystic fibrosis [10]. The average incidence of SMA is 1:6,000–1:10,000 live births and the carrier frequency is 1:40–1:60 [10, 11].
There are no published epidemiological data on SMA in Estonia and the other Baltic countries. The aim of this study was to estimate the incidence of SMA I in Estonia.

**Method and Patients**

This is a population-based descriptive epidemiological study involving the whole of Estonia. A retrospective and prospective study was conducted spanning the period from January 1994 to December 2003. The analysis was based on case histories collected from the children's units of the two tertiary hospitals in Estonia – Children's Clinic of Tartu University Hospital and Children's Hospital in Tallinn. According to the existing guidelines all children under the age of 18 years in Estonia with any developmental problems (including suspicion of SMA and other neuromuscular disorders) were included in the study. The diagnosis was established on the basis of neurological evaluation, electroneuromyographic (ENMG) findings, DNA analysis and muscle biopsy taking into consideration the whole of Estonia. A retro- and prospective study was conducted spanning the period from January 1994 to December 2003. The analysis was based on case histories collected from the children's units of the two tertiary hospitals in Estonia – Children's Clinic of Tartu University Hospital and Children's Hospital in Tallinn. According to the existing guidelines all children under the age of 18 years in Estonia with any developmental problems (including suspicion of SMA and other neuromuscular disorders) were included in the study. The diagnosis was established on the basis of neurological evaluation, electroneuromyographic (ENMG) findings, DNA analysis and muscle biopsy taking into consideration the whole of Estonia.

Before the study started several courses for doctors in these hospitals were organized about the clinical symptoms, the course of the disease and study protocol. After the implementation of genetic diagnostics in Tartu University Hospital in 1994, information about the genetic diagnostics of these neuromuscular disorders were also included in the advanced courses for family doctors and pediatricians. All patients with SMA I born and diagnosed in the period January 1994 to December 2003 were included in the study. The diagnosis was established on the basis of neurological evaluation, electroneuromyographic (ENMG) findings, DNA analysis and muscle biopsy taking into account the SMA diagnostic criteria [2]: age of onset before the age of 6 months in SMA I; muscle weakness and wasting of the trunk and limbs, symmetrical, lower limbs more than upper, proximal more than distal; fasciculation of tongue, tremor of hands; on ENMG abnormal spontaneous activity (fibrillations, fasciculations), increased mean duration and amplitude of motor unit action potentials; in muscle biopsy atrophic fibers of both types, hypertrophic fibers of type I, type grouping; C, CK levels <5 times the upper limit of normal; the homozygous absence or mutation of the SMN1 gene. Molecular analysis of the SMN genes involved amplification of exon 7 followed by restriction with the HindIII enzyme and amplification of exon 8 where the PCR product was cleaved by the DdeI enzyme [12]. Figure 1 shows an example of PCR restriction analysis of SMA patients. The homozygous deletion of the SMN1 gene can be clearly identified as the SMN1 exon 7 product contains two HindIII sites and the SMN2 exon 7 contains one HindIII site.

Birth incidence was defined as the number of individuals born in the study region during a specific time period that developed the disease in relation to the total number of live-born babies during the same period and region. The number of live births in Estonia during that 10-year period was 129,832 babies and at January 1, 2004 the population of Estonia was 1,351,069 of whom 622,450 were males and 728,619 were females. Data about live births were obtained from the authorized statistics of the Estonian Statistical Office (www.stat.ee). The birthrate in Estonia has decreased considerably during the last decade.

The study was approved by the Ethics Committee of Human Studies of Tartu University. Informed consent for the medical procedures including genetic tests was obtained from the parents.

**Results**

A total of 15 cases of SMA were identified during a 10-year period from January 1994 to December 2003, of those 9 were diagnosed as SMA I and confirmed by molecular testing (Table 1).

Apart from these 9 cases, one more male patient was identified with somewhat similar clinical signs but in his case the diagnosis of SMA I could not be confirmed by molecular testing. As he also had additional symptoms that are not considered part of the classical SMA phenotype, he was excluded from the study.

All patients were from different families and none of them had a positive family history of SMA. There was only one girl among the 9 patients diagnosed with SMA I during that time. In 6 cases, the symptoms were present at birth and the confirmed diagnosis of SMA I was made during the first week of life. The other 3 cases were also diagnosed before the age of 3 months.

Two of the children were alive at December 31, 2003, both born in 2003 (patients 8, 9). Among the remaining 7 cases the longest survival time was 1 year and 6 months and the shortest was only 20 days. The mean age at death was 8 months.
Molecular investigation revealed deletions in the SMN1 gene in all the 9 cases. Homozygous deletion of exons 7 and 8 of the SMN1 gene was found in 8/9 cases, 1/9 had only exon 7 deleted. In case the first result was negative, the analysis was repeated. The SMN2 gene copy number was not investigated.

ENMG studies in all 9 patients showed lesions at the level of the motor neuron in the spinal cord. Muscle biopsy was performed on one child only, because the first DNA analysis did not find the deletion in the SMN1 gene, and the findings supported the diagnosis of SMA (patient 4). Later the DNA analysis was also repeated proving the presence of deletion in the SMN1 gene.

One child (patient 5) had congenital bone fractures and heart defect as additional features that are usually not considered part of the classical SMA phenotype.

### Discussion

This is the first population-based epidemiological study on SMA in Estonia and in the Baltic countries. We found the incidence of SMA I in Estonia to be 1 in 14,400 live births. Our incidence rate is lower than the average incidence rate around 1:10,000 live births for the world. It is similar to the results from Hungary of 1:15,152 live births [13]. However, even lower incidence rates have been found in Poland (1:19,474 live births) [14], England (1:25,708 live births) [15], and Italy (4.1:100,000 live births) [16]. In Europe, higher rates of SMA have been reported in Northern Finland (1:7,100 live births) and Slovakia (1:5,600 live births) [17].

One shortcoming of our study is the small number of cases. However, it covers the whole population under the age of 18 years and can be explained by the overall small population in Estonia. At the same time, based on the existing guidelines and the experience with other rare disorders in Estonia, we are quite confident that we have found all the cases of SMA I. A special programme to define different hypotonic syndromes was carried out during 2003–2005 with similar results [unpubl. data].

In our study 6 of the SMA I patients were of Estonian origin and 3 of Slavic origin. The distribution of patients of Estonian and Slavic origin reflects Estonia’s demographic characteristics, showing no difference between Estonians and the people of Slavic origin.

Homzygous deletion of exons 7 and 8 of the SMN1 gene is found in 90–98% of SMA I patients [5, 6]. Mutation of the SMN1 gene is also one of the diagnostic criteria of the disease. The overall frequency of SMN1 deletions among our SMA I patients was 100%. SMN1 deletion has been found in 96% of SMA I patients in Germany [18], in 93% of SMA patients from the Netherlands [5]. Homozygous deletion of exon 7 of SMN1 was found in 96.2% of SMA I patients in Slovakia [17]. Most of the patients with SMA I show signs of the disease by the age of 3 months or 6 months at the latest [2]. All cases in our study were diagnosed before that age. Even more, 6 children (6/9) showed signs of the disease at birth and all of them needed immediate intensive care. The diagnosis was confirmed by the third week of life at the latest.

Survival of the children with SMA I beyond the age of 2 years is rare and 80% of children die within the first year [19]. About 95% of SMA I patients die before the age of 18 months and 50% are dead by the age of 7–8 months [18]. In our study not one child survived beyond 18 months, 70% were dead by the age of 7 months, explained by the severity of the cases.

<table>
<thead>
<tr>
<th>No.</th>
<th>Year of birth</th>
<th>Sex</th>
<th>Age at onset of symptoms months</th>
<th>SMN1 deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1994</td>
<td>male</td>
<td>0</td>
<td>7, 8 exon deletion</td>
</tr>
<tr>
<td>2</td>
<td>1996</td>
<td>male</td>
<td>0</td>
<td>7, 8 exon deletion</td>
</tr>
<tr>
<td>3</td>
<td>1997</td>
<td>male</td>
<td>1</td>
<td>7, 8 exon deletion</td>
</tr>
<tr>
<td>4</td>
<td>2000</td>
<td>male</td>
<td>0</td>
<td>7, 8 exon deletion</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>female</td>
<td>0</td>
<td>7, 8 exon deletion</td>
</tr>
<tr>
<td>6</td>
<td>2001</td>
<td>male</td>
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<td>7, 8 exon deletion</td>
</tr>
<tr>
<td>7</td>
<td>2002</td>
<td>male</td>
<td>0</td>
<td>7, 8 exon deletion</td>
</tr>
<tr>
<td>8</td>
<td>2003</td>
<td>male</td>
<td>2.5</td>
<td>7 exon deletion</td>
</tr>
<tr>
<td>9</td>
<td>2003</td>
<td>male</td>
<td>1</td>
<td>7, 8 exon deletion</td>
</tr>
</tbody>
</table>

Table 1. SMA I patients in 1994–2003

SMA I in Estonia
It is complicated to explain the sex distribution among our group of SMA I patients but male predominance has also been reported earlier [20]. One reason for this uneven sex distribution in our study could be the small number of cases. The only girl diagnosed with SMA I during the study period had a very severe course of the disease. In her case the decrease in fetal movements was noticed about 3 weeks before birth. She had long bone fractures and an additional heart defect. It is possible that girls are more severely affected with the onset of the disease already in utero and these children are stillborn. Unfortunately, stillborn babies are not investigated for SMA diagnosis during autopsy, but this would probably bring some explanation to the differences in the incidence rates between sexes. During the next 2 years after the end of this study 2 new cases of SMA I were diagnosed in 2004 (both girls) and 1 case in 2005 (a boy).

In the literature, 6 patients with SMA and congenital fractures have been described before. Five of those were male infants and X-linked inheritance could not be excluded in these cases [21–24]. One was a female who showed no deletion of the SMN1 gene [25]. Our patient with congenital fractures was a female and had exons 7 and 8 of the SMN1 gene deleted.

It is interesting to stress that only 2 cases were diagnosed in the Children’s Hospital in Tallinn where patients from the northern part of Estonia are referred. This suggests that an uneven distribution of the SMA gene may exist or are all the severe cases coming to the University Hospital in Tartu. Some differences in gene distribution in Estonia have been shown in some other genetic disorders, e.g. phenylketonuria [26]. The incidence rate of phenylketonuria during 1993–1998 was 1:6,018 live births (total 80,523 live births) [27] and the incidence rate of cystic fibrosis during 1974–2003 was 1:7,743 live births (total 588,502 live births) [28]. These two disorders are about two times more frequent in Estonia than SMA I.

We conclude that the incidence of SMA I of 1:14,400 in Estonia is lower than the worldwide incidence rate but similar to some Eastern European countries (Hungary, Poland). The male predominance among the patients needs further research including the investigation of the stillborn babies for SMA I diagnosis. The incidence rates of relatively rare genetic syndromes are important in planning the proper health care.

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