Molecular diagnosis of spinal muscular atrophy in Egyptians

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استخدام التحاليل الجزيئية في تشخيص الضمور العضلي النخاعي المنشأ عند المصريين

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خلاصة: أُجريت هذه الدراسة على ثلاثة وثلاثين مريضاً بالضمور العضلي النخاعي المنشأ. ولقد تمت التحاليل الجزيئية الدناوية للجين المسبِّب للضمور العضلي النخاعي والموجود على الذراع الطويلة للصبغي رقم 5 (5q11.2q13.3)، وكشفت عن فقدان زيجوتي متماثل للإكسون رقم 7 في 55% من الحالات، بينما كان لدى 36% من هؤلاء فقدان زيجوتي متماثل للإكسون رقم 8. وكان المرضى البالغون متغايري الزيجوت من حيث الحجم الشاذ للإكسون رقم 8. أما المرضى الباقون فقد كان لديهم فقدان مركّب متغاير الزيجوت للإكسونَيْن رقمي 7 و8 أو أنهم كانوا أسوياء الإكسونَيْن. وبناء عليه، يمكن أن يكون هناك ضمور عضلي نخاعي المنشأ غير مرتبط بالذراع الطويلة للصبغي رقم 5 أو ضمور عضلي نخاعي المنشأ ناجم عن طفرات أخرى. إن اكتشاف فقدان الإكسونَيْن رقمَيْ 7 و8 في حالات الضمور العضلي النخاعي المنشأ هو اختبار تشخيصي قوي في المصابين بهذا الضمور. ولكن يجب أيضاً البحث عن طفرات أخرى لدى المصريين.

ABSTRACT This study was carried out with 33 spinal muscular atrophy (SMA) patients. DNA molecular studies of the SMA gene on the long arm of chromosome 5 (5q11.2q13.3) revealed homozygous deletion of exon 7 in 55% of cases, 36% of whom also had a homozygous deletion of exon 8. The adult patients were heterozygous for an abnormal size exon 8. The remaining patients had either compound heterozygote deletion of exons 7 and 8 or were normal for both. There may therefore be 5q unlinked SMA or SMA due to other mutations. Detection of deletions of SMA exons 7 and 8 is a powerful diagnostic test in patients with SMA, but other mutations among Egyptians must also be sought.

Diagnostic moléculaire de l'amyotrophie spinale chez des Egyptiens

RESUME Cette étude a été réalisée sur 33 patients atteints d'amyotrophie spinale. Les études moléculaires de l'ADN pour le gène de l'amyotrophie spinale sur un long bras du chromosome 5 (5q11.2q13.3) ont révélé une délétion homozygote de l'exon 7 dans 55 % des cas, dont 36 % avaient une délétion homozygote de l'exon 8. Les patients adultes étaient hétérozygotes pour un exon 8 de taille anormale. Les patients restants avaient une délétion hétérozygote mixte des exons 7 et 8 ou étaient normaux pour les deux. Il peut donc y avoir des amyotrophies spinales non liées à 5q ou dues à d'autres mutations. La détection des délétions des exons 7 et 8 d'amyotrophie spinale est une bonne épreuve diagnostique chez les patients atteints d'amyotrophie spinale, mais d'autres mutations chez les Egyptiens doivent également être recherchées.

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Introduction

Spinal muscular atrophy (SMA) is a clinically and genetically heterogeneous group of diseases characterized by degeneration and loss of the anterior horn cells in the spinal cord and sometimes also in the brain stem nuclei, resulting in muscle weakness and atrophy. The progressive denervation of muscles is compensated in part by re-innervation from an adjacent motor unit, but giant motor units are thus created with subsequent atrophy of muscle fibres when the re-innervating motor neurons eventually become involved. The sensory neurons are spared and there are no signs of upper motor neuron pyramidal tract involvement [*1*].

Environmental influences have little effect on the clinical manifestations of this group of diseases. Effects of social class, birth order, parental age, sex and disease clustering in time and place have all been investigated and found to be inconsequential in comparison with the effects of the basic genetic defect [*2*]. In SMA patients, life expectancy may vary from only a few months in the case of SMA type I to a normal life expectancy in the case of distal SMA due to an autosomal dominant gene [*3*]. SMAs are incurable, thus making genetic and prognostic considerations of great importance. Both affected patients and normal parents who have SMA children and are making reproductive decisions require three key pieces of information:

- an accurate diagnosis not only of the clinical type of SMA involved but also of the specific causal gene, e.g. autosomal recessive adult onset SMA
- the recurrence risk involved
- a knowledge of the natural history of the form of SMA in question, including the full implications of its medical management and potential complications.

The subdivision of SMAs into separate genetic and clinical entities is controversial unless biochemical or molecular genetic criteria are available to define distinct pathogenic mechanisms. The criteria used are age at onset, severity (progression, age at death), distribution of weakness, inclusion of additional features and different modes of inheritance. With recent molecular genetic advances in detecting deletions on the long arm of chromosome 5, it is now possible to confirm the diagnosis of proximal SMA with onset in childhood or youth [*4*].

The SMA gene is located in a small region of the long arm of chromosome 5 (5q11.2q13.3) that contains many repeated sequences, including several genes and markers. Three candidate genes have been isolated and shown to be deleted in SMA patients: the survival motor neuron gene *(SMN)*, the neuronal apoptosis inhibitory protein gene *(NAIP)*, and the X S2G3 cDNA. The *SMN* gene is encoded by two nearly identical sequences: the centromeric SMN^C and the telomeric SMN^T. Both of these are transcribed and can be distinguished by two single nucleotide sequence variations in exons 7 and 8 [*5*]. Deletions of *NAIP* exon 5 and of *SMN* exon 7 have been associated with a 5-fold increased risk of type I SMA [*6*]. The aim of our study was to identify mutations involving the SMA gene in Egyptian patients with proximal SMA, so that prenatal and presymptomatic diagnosis in informed families can be performed. Correlation between genotype and phenotype was also carried out.

Methods

The study involved 33 families with one or more members with SMA followed up in the Outpatient Genetics Clinic, Paediatric Department, Ain-Shams University Hospital. The sample included 33 index patients with SMA and 10 normal controls. The age of the child patients at the time of the study ranged from 1.5 months to 6 years, and there were 3 adult patients aged 30–35 years. There were 15 males and 18 females. The sample was divided into three groups according to the age of onset and rate of progression of the disease.

- Group I, acute infantile SMA: age of onset before 6 months and rapidly progressive. This group included 21 patients, 12 females and 9 males.
- Group II, chronic childhood SMA: onset after the age of 6 months and slowly progressive. This group contained 9 patients, 6 females and 3 males.
- Group III, adult type: onset in the third decade of life and showing slow progression. This group included 3 male patients.

All the patients had a complete history taken with emphasis on age at onset, progress of the disease, perinatal history, consanguinity and family history of similar conditions or other genetic data. A pedigree was constructed to identify other affected members in the family. The patients also had a thorough clinical examination focussing on neurological parameters, tone, power, reflexes, wasting and atrophy of muscles, abnormal movements and sensations. Other investigations included serum creatine phosphokinase (CPK), electromyogram (EMG) and nerve conduction velocity, and DNA molecular studies for affected members and controls using single-stand conformation polymorphism analysis [7].

Results

Sluggish fetal movements and kicking *in utero* were reported by the mothers of 21 patients. Other details of the prenatal, natal and postnatal history were found to be irrelevant. A history of a similar condition was present in 18 families (55% of cases). First cousin consanguinity was present in 15 families (45% of cases).

The most common complaints of the patients were repeated chest infections (6 patients), hypotonia and weakness with delayed motor milestones (21 patients), and secondary inability to walk (6 patients). The parents noticed manifestations of the disease at the age of 1 month in 3 patients, at 2 months in 9 patients, at 4 months in 9 patients, and after 6 months in another 9 patients. For the 3 adult patients, onset was in the third decade of life.

The disease was rapidly progressive in 21 patients, all of whom died of respiratory failure aged between 3 months and 1.5 years. The disease was slowly progressive in the remaining 12 patients; 3 of whom died at the age of 2.5 years of respiratory failure, and the rest are still alive.

All patients presented with weakness, hypotonia and wasting of muscles. Weakness and hypotonia were profound in the first group, the infantile SMA, but wasting was not marked because of early death; deep reflexes were absent, sensations were intact and mentality was normal. Tongue fasciculations were prominent in all patients and hand tremors were present in the second and third groups of patients.

Table 1 **Genotype of the patients with spinal muscular atrophy**

Patient	Age at presentation	Sex	Exon 7 amplification	Copy gene *Dra I*	Genotype	Exon 8 amplification	Copy gene *Dde I*	Genotype	Remarks
1–3	2 y	Male	+	# (1)	O/O	+	# (2)	O/O	
4–6	1 y	Female	+	# (1)	O/O	+	# (2)	N/N or N/O	
7–9	10 m	Female	+	# (1)	O/O	+	# (2)	O/O	
10–12	50 d	Female	+	# (1)	N/N or N/O	+	# (2)	N/N or N/O	Possibly other exons involved
13–15	6 y	Male	+	# (1)	N/N or N/O	+	# (2)	N/N or N/O	Probably due to other exon deletions or 0–0 in trans.
16–18	5 m	Female	+	# (1)	O/O	+	# (1)[a]	N/N or N/O	
19–21	8 m	Male	+	# (1)	O/O	+	# (2)	O/O	
22–24	3.5 m	Female	+	# (1)	N/N or N/O	+	# (2)	N/N or N/O	Probably due to other exon deletions or 0–0 in trans.
25–27	8 m	Female	+	# (1)	O/O	+	# (2)	O/O	
28–30	6 m	Male	+	# (1)	N/N or N/O	+	# (2)	N/N or N/O	Other exon deletions or 0–0 in trans.
31–33	30–35 y	Male	+	# (1)	N/N or N/O	+	# (2)	N/A	

[a] *No adequate explanation.*
+ = amplification was successful or restriction digestion was successful.
N = normal gene or exon or other mutation.
Dra I, Dde I = *restriction enzymes.*
Copy gene = pseudogene.
O = absent gene or exon.
 # = fragments produced by restriction digestion.
A = abnormal size allele or exon.

Table 2 **Exons 7 and 8 status in patients with spinal muscular atrophy**

Genotype	No. of patients	
	Exon 7	Exon 8
Patients with homozygous deletion (O/O–O/O)	18	12
Patients with compound heterozygous or normal (N/O–N/N)	12	18
Patients with heterozygous or normal exon 7 and abnormally sized exon 8 (N/O or N/N–N/A)	3	3

O = gene or exon absent.
N = normal exon 7 or 8 (probably other exons affected or other mutations present).
A = abnormally sized allele or exon.

Table 3 **Distribution of patients with spinal muscular atrophy by clinical severity and molecular structure**

Clinical severity	Homozygous deletion of both exons 7 and 8 (O/O–O/O)	Homozygous deletion of exon 7 with either heterozygous or normal exon 8 (N/O–N/N)	Double heterozygous deletion of both exons 7 and 8 (N/O–N/O)	Heterozygous exon 7 and abnormal exon 8 (N/O–N/A)
Type I (severe)	9	6	6	–
Type II (moderate)	3	–	6	–
Type III (adult)	–	–	–	3

O = gene or exon absent.
N = normal exon 7 or 8 (probably other exons affected or other mutations present).
A = abnormally sized allele or exon.

CPK was normal or slightly elevated in all patients. EMG showed a neurogenic pattern of affect, while nerve conduction velocity was normal. Muscle biopsy revealed large group atrophy in severe SMA, but the atrophic group of both fibre types appeared to be smaller in the chronic form of the disease.

Molecular studies (Tables 1–3) showed 18 patients (55%) to have homozygous deletions of exon 7, 12 of whom (36%) also had homozygous deletions of exon 8. All these patients had SMA types I or II. The other 12 patients (36%) had either a heterozygous deletion of both exons 7 and 8, or both exons were normal. These patients also belonged to SMA types I and II. Three patients (9%) were either heterozygous or normal for exon 7 and heterozygous for an abnormally sized atypical exon 8 (adult type SMA).

Discussion

In our study, 21 mothers of SMA type I patients noted sluggish fetal movements during pregnancy. This demonstrates that

the manifestations of SMA may be apparent *in utero* [*3*]. These patients died before the age of 1.5 years with type I SMA (Werdnig–Hoffmann or acute infantile SMA). The earlier the age of onset of clinical manifestations, the earlier the age of death. There were also 9 patients with age of onset after 6 months (type II SMA, arrested Werdnig–Hoffmann or intermediate SMA); 3 of these died aged 2.5 years, while the other 6 are still alive. The adult cases, who developed secondary inability to walk with severe hypotonia, wasting, tongue fasciculations and hand tremors, are still alive (type III or adult SMA).

There was a predominance of females to males, 6:5. In contrast, a study by Zerres and Rudnik-Schoneborn showed a predominance of males that was most pronounced in the mild form [*4*]. These authors suggested that there may be a female sparing factor, responsible for the marked decrease in the number of affected girls in the late onset group. However, no significant sex effect was established by the study done by Rudnik-Schoneborn et al. [*8*].

Consanguinity was present in 45% of our patients, whose parents were first cousins. This percentage is higher than the average reported among Egyptians (28%) [*9*]. These data reveal the importance of lowering the consanguinity rate and the value of genetic counselling and prenatal diagnosis in preventing SMA in our community.

Eighteen families (55%) had a positive history of similarly affected family members. A report from Hungary gave a rate of 32% [*10*]. In our familial cases, the age of onset was very similar and all the affected family members belonged to the same SMA type. However, another study [*8*] suggested that other SMA types such as I and III can frequently be observed among the siblings of patients with SMA type II, another argument in favour of a continuous spectrum in childhood SMA.

The mode of inheritance in our cases was autosomal recessive, which is enhanced by a high consanguinity rate. Becker suggested an allelic model for SMA, in which there are three or more normal alleles (a, a', a") in addition to the pathological gene a(+) [*11*]. The genotype a' a(+) was thought to lead to the Kugelberg–Welander phenotype (type III) and the a" a(+) genotype to the Werdnig–Hoffmann phenotype (type I). However, Muller et al. presented evidence contradicting Becker's hypothesis [*12*]. In their study, the segregation of linked markers indicated that the same alleles were involved.

EMG and muscle biopsy were performed on all our patients and were diagnostic, as indicated by Aminoff [*13*] and Banker [*14*].

Of the 33 patients, molecular studies revealed 18 patients (55%) with homozygous deletions of exon 7, 12 (36%) of whom also had a homozygous deletions of exon 8. The other 6 patients (18%) had homozygous deletions of exon 7 and either heterozygous deletions of or a normal exon 8. All of these patients were types I and II SMA. Thompson et al. [*5*] detected homozygous deletions of exons 7 and 8 in 58% of cases and Burlet [*15*] reported homozygous deletions of exons 7 and 8 in 43% of cases. The remaining 12 patients (36%) in our study had either heterozygous deletions of exon 7 in one chromosome and another mutation in the other chromosome, or normal exons 7 and 8 combined with other mutations in the *SMN* gene in both chromosomes, or they were chromosome

5 unlinked. The three patients with adult SMA (9%) had either heterozygous deletions of exon 7 or normal exon 7, together with a heterozygous abnormal exon 8.

In terms of genotype–phenotype correlation, 9 patients with the severe type I SMA had homozygous deletions of both exons 7 and 8. Roy et al. [*16*] found mutations in a gene encoding *NAIP* in the proximal portion of the critical SMA region of 5q13.1 in cases of severe SMA, leading to failure of the normal inhibition of motor neuron apoptosis that resulted in or contributed to the SMA phenotype. Another 6 patients with the severe type I SMA had homozygous deletions of exon 7 and either normal or heterozygous deletions of exon 8. Since the SMN^T is completely deleted, the remaining SMN^C gene cannot produce a full-length protein to compensate [*17*]. The remaining 6 patients with the severe type I SMA were either doubly heterozygous for deletions of exons 7 and 8, or were normal for both of them. If they were compound heterozygotes, they may have had other mutations of *SMN*. The other possibility for patients with intact exons 7 and 8 is that they were chromosome 5 unlinked, as with severe neonatal SMA with diaphragmatic paralysis [*18*].

Three patients with type II SMA had homozygous deletions of both exons 7 and 8. Wirth et al. explained this by the presence of the marker *AgI-CA* which correlated with phenotype [*19*]. One copy of the marker on both chromosomes, or a 1,1 genotype, is more frequent in type I SMA, whereas a 1,2 genotype is more frequent in type II SMA. Six patients with moderate type II SMA had either heterozygous deletions of both exons 7 and 8 or were normal for both. This may be explained by the presence of other mutations, such as a 4 bp deletion in exon 3 and one allele of the critical isoform with sufficient skipping to produce some product [*20*]. The adult patients had either heterozygous deletions of or a normal exon 7, and a heterozygous abnormal exon 8. Recently, genetic homogeneity between childhood onset and adult onset autosomal recessive SMA was reported, as identical deletions were discovered in the patients investigated [*21*].

Using a competitive strategy to determine the SMN^T and SMN^C gene copy number, it is possible to identify SMA carriers and to distinguish between non-5q SMA-linked patients and compound heterozygote-5q SMA patients. Analysis of normal and carrier individuals by this assay clearly indicates that the copy number of SMN^T and SMN^C varies from zero to at least 2 per chromosome, and that the majority of SMA carriers have a single copy of the SMN^T gene on their normal chromosome [*22*].

To conclude, detection of deletions of *SMN* exons 7 and 8 is a powerful diagnostic test in patients with SMA. This test can detect all patients with homozygous deletions but is not quantitative. It cannot detect heterozygous deletions nor distinguish 5q-unlinked SMA patients and compound heterozygotes. Accurate dosage analysis must be performed. We should continue to search for other mutations within the Egyptian population, to facilitate the detection of carriers and presymptomatic cases and for prenatal diagnosis.

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Announcement

The Fifth Saudi School on Human Molecular Genetics will be held from 18-25 Shaban, 1423 AH (24-31 October 2002 AD). The deadline for submission of applications is 30 July 2002 of from abroad and 1 October 2002 if local.

A symposium entitled: Genetics in Health and Disease - Status, Implicit and Implications for Individuals and Community and the 3rd MEGA Meeting will be held from 27-29 Shaban 1423 AH (2-4 November 2002 AD). The deadline for submission of abstracts is 1 October 2002. The deadline for registration and visa application from abroad is 15 August 2002 and for pre-registration from within the country is 15 October 2002.

Further information on both these meetings can be obtained from the Organizing Committee, Department of Medical Biochemistry & WHO Collaborating Centre, Postgraduate Centre, College of Medicine, King Saud University, PO Box 2925, Riyadh 11461, Saudi Arabia. Telephone: (966) 1 4670831/ 4671551; Fax: (966) 1 4672575/4811853; E-mail: mohsen@ksu.edu.sa