Expansion of CAG repeats in the spinocerebellar ataxia type 1 (SCA1) gene in idiopathic oligozoospermia patients

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Abstract

Purpose The lengths of CAG repeats in two spinocerebellar ataxia genes, SCA1 and SCA3, were analyzed to determine whether such repeats exist in higher numbers in infertile males.

Methods Blood samples were collected from healthy controls, oligozoospermia patients, and azoospermia patients. DNA fragments containing target CAG repeats were amplified by PCR with template DNA purified from the blood samples. CAG repeats in PCR fragments were determined, using ABI PRISM 310 Gene Analyzer.

Results In SCA1, the distribution of CAG repeats in oligozoospermic males was different from that of the control group: More alleles had a repeat number that exceeded 32. Conversely, for SCA3, the examined oligozoospermia and azoospermia patients exhibited no differences in distribution of CAG repeats in comparison with the control group.

Capsules The spinocerebellar ataxia type 1 (SCA1) gene in a subset of idiopathic oligozoospermia patients showed increased numbers of CAG repeats.

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Conclusions SCA1 in a subset of oligozoospermia patients has an increased number of CAG repeats.

Keywords CAG repeats · Oligozoospermia ·
Spinocerebellar ataxia type 1 gene ·
Spinocerebellar ataxia type 3 gene

Introduction

The recent development of intracytoplasmic sperm injection (ICSI) enables pregnancy to be achieved by using sperm from oligospermic, asthenospermic, and teratospermic men, as well as spermatid from azoospermic men [1]. As ICSI circumnavigates infertility, it raises the concern that a natural preventive method against genetically lethal inheritance from father to child has been eliminated [2].

Trinucleotide repeats are commonly observed in the human genome. Although these trinucleotide repeats are polymorphic and their number varies among individuals, the lengthening of these repeats is associated with many genetic diseases [3]. For example, longer CAG repeats in the androgen receptor gene are associated with male infertility, endometrial carcinoma [4, 5], BRCA-1-associated breast cancer and male breast cancer [6, 7]. A correlation has also been established between the length of CAG repeats in mitochondrial DNA polymerase gamma gene and male infertility [8], although this finding was inconsistent with that of another study [9]. Spinocerebellar ataxia is also associated with spinocerebellar ataxia type 1 (SCA1) gene with increased trinucleotide repeat numbers from 39 to 82 in ataxin-1 protein (6–44 in normal controls), as well as with spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph Disease (MJD) gene with increased trinucleotide repeat numbers from 55 to 84 in ataxin-3 protein (12–40 in normal controls) [10]. Cummings

et al demonstrated that ataxin-1 with an expanded polyglutamine track is often misfolded, making it a target for proteasome degradation [11]. Moreover, proteins with an expanded polyglutamine track often aggregate with caspase to cause apoptosis [12].

Some studies have established that the anticipation is typically high for spinocerebellar ataxis and Machado-Joseph disease [13]. Since the length of the CAG repeats in a gene may increase as they are transmitted to the next generation [13], genetic instability of trinucleotide repeats contributes to anticipation. For instance, CAG repeats in SCA1 tend to lengthen during spermatogenesis, explaining why the severity of the disease frequently increases from one generation to the next [14]. Additionally, the anticipation in spinocerebellar ataxia exceeds that of bulbar muscular atrophy [14]. In this study, we screened CAG repeats in SCA1 and SCA3 genes from 31 oligozoospermic patients, 56 azoospermic patients and 35 healthy controls, to determine whether infertile males possess longer CAG repeats in these two loci.

Materials and methods

Patients

Patients screened for this study included infertile men aged 25 to 40. The study included 31 subjects in the oligozoospermia group with sperm counts less than 20×10^6 spermatozoa/ml. The azoospermia group, in which there was no sperm observed on semen analysis, contained 56 subjects. Patients in both groups were examined by urologists and confirmed to have no obstructive or explanative causes for abnormal sperm counts. Thirty-five individuals with normal sperm counts were also enrolled, regardless of their parental status. The Institutional Review Board of Chung Shan Medical University Hospital approved all procedures, and informed consent was obtained from all subjects prior to collecting their genetic material for the study.

Isolation of DNA from blood

DNA was purified from peripheral blood lymphocytes using a previously described method [15]. The DNA was finally dissolved in 100 μ l of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA).

Polymerase chain reaction

The DNA fragments of SCA1 and SCA3 that contained CAG repeats were amplified [16, 17]. A slight modification was made in PCR primers and probes for SCA1 and SCA3 as follows: SCA1-F, 5'-CAACATGGGCGAGTCTGAG-3';

SCA1-R, 5' HEX-AACTGGAAATGTGGACGTAC-3'; SCA3-F, CCAGTGACTACTTTGATTCG-3'; SCA3-R, 5' FAM-GGTGGCTGGCCTTTCACATGGAT-3'. Each amplification reaction mixture (25 μ l) consisted of 200 nM primers and probes, 200 μ M dNTP, 2.5 μ l 10 $\times$ PCR buffer containing 20 mM MgCl₂, and 1.25 units of VioTaq Taq DNA polymerase (Viogene, Calif., USA). The reaction mixture was initially incubated at 94°C for 5 min, followed by 28 cycles at 94°C for 1 min, 58°C for 1 min and 72°C

Fig. 1 CAG repeats in SCA1 from oligozoospermia and azoospermia patients. DNA fragments that contained CAG repeats in SCA1 from 35 individuals with normal sperm counts (Normal) (a), 31 oligozoospermia patients (b) and 56 azoospermia patients (c) were amplified by PCR and analyzed by ABI PRISM 310 Gene Analyzer

Table 1 CAG repeats in SCA1 and SCA3 genes

Group	SCA1			SCA3		
	Control	Oligozoospermia	Azoospermia	Control	Oligozoospermia	Azoospermia
Allele range (in repeats)	23–35	19–38	9–68	6–45	6–36	13–35
Total number of alleles	70	62	112	70	62	112
Observed heterozygosity (%)	85.7	100	78.6	77.1	83.9	89.3
Allele with maximum frequency						
Allele (in repeats)	29	30	29	14	14	14
Frequency (%)	34.3	21.0	29.5	41.4	41.9	33.0
Mean (SEM)	29.4 (2.04)	30.4 (3.10)	29.6 (4.48)	20.7 (7.64)	20.1 (6.08)	21.4 (6.63)
Median	29	30	29	19	19	21.5
p^a	–	0.030*	0.831	–	0.766	0.387

^a Differences between the control and oligozoospermia or azoospermia groups were assayed by the Mann-Whitney U-test.

* <0.05 , statistically significant

SEM: standard error of the mean

for 2 min in a thermal cycler. After the final amplification cycle, DNA extension was performed at 72°C for 10 min.

Analysis of PCR fragments

The fluorogenic PCR product (1 μ l) was added to a solution that contained 11.5 μ l Hi-Di formamide and 0.5 μ l Tamra size standard (Applied Biosystems, Calif., USA). The samples were incubated at 95°C for 5 min and then examined with ABI PRISM 310 Gene analyzer (Applied Biosystems, Calif., USA). The GeneScan Analysis 3.12 (Applied Biosystems, Calif., USA) software was used to analyze the experimental results.

Statistics

The Mann-Whitney U-test was used to evaluate the differences in CAG repeat distributions in SCA1 and SCA3 among the three groups. The differences in the frequencies of larger alleles in each gene were further tested by Chi-Square test. $P<0.05$ was considered significant.

Results

Analysis of the CAG repeats in SCA1 revealed that 70 alleles from 35 healthy men in the control group had 23 to 35 repeats with an observed heterozygosity of 85.7% (Fig. 1a and Table 1). Of these alleles, 24, or 34.3%, had 29 copies of CAG trinucleotides. Most of the alleles (68 alleles representing 97.1% of the population) had 23 to 32 copies of the CAG sequence in the CAG track. Only two alleles (2.9% of the population) from two individuals had 35 CAG repeats. CAG repeats in SCA1 from 31 oligozoospermic males showed a different distribution (Fig. 1b and Table 1). The repeat numbers ranged from 19 to 38 with 100% heterozygosity. The most observed repeat number was 30, in 21.0% of the alleles examined. Among the 62 alleles, 10 (16.1%) contained more than 32 CAG trinucleotides, with the number ranging from 33 to 38. The median number of CAG repeats was 30, which exceeded the median number of 29 of the control group ($p=0.03$). Statistical analysis also revealed that the frequency of CAG repeats longer than 32 in SCA1 was significantly

Table 2 Comparison of longer CAG repeats in SCA1 and SCA3 genes

Gene	CAG repeats	Control	Oligozoospermia		Azoospermia	
		Allele number	Allele number	χ^2 (p) ^a	Allele number	χ^2 (p) ^s
SCA1	9–32	68	52	7.008 (0.008*)	110	0.230 (0.631)
	>32	2	10		2	
SCA3	6–29	65	57	0.040 (0.842)	103	0.048 (0.826)
	>29	5	5		9	

^a Differences between the control and oligozoospermia or azoospermia groups were determined by Chi square analysis.

* <0.05 , statistically significant

Fig. 2 CAG repeats in SCA3 from oligozoospermia and azoospermia patients. DNA fragments that contained CAG repeats in SCA3 from 35 individuals with normal sperm counts (Normal) (a), 31 oligozoospermia patients (b) and 56 azoospermia patients (c) were amplified by PCR and analyzed by ABI PRISM 310 Gene Analyzer

higher in oligozoospermia patients than in the control group ($p=0.008$) (Table 2). Among the 112 alleles from 56 azoospermia patients, the numbers of CAG repeats ranged from 9 to 68 with a heterozygosity percentage of 78.6 (Fig. 1c and Table 1). The median number of CAG repeats, 29, equaled that of the control group ($p=0.831$). The most frequently observed number of repeats was 29, which was present in 29.5% of the patients. Unlike the oligozoospermic subjects, around 98.2% of the azoospermic males had 32 or fewer CAG repeats. Only two alleles (1.8%) from two

patients had a higher number of CAG repeats—37 and 68, respectively.

CAG repeats in SCA3 were also analyzed (Fig. 2 and Table 1). The number of CAG repeats in healthy males varied widely from 6 to 45. The oligozoospermia patients had 6 to 36 copies of CAG trinucleotides and azoospermia patients had 13 to 35 copies. The most common number of repeats was 14. However, many alleles had a repeat number of 26 or 27. Statistical analysis did not reveal any difference in the number of repeats in SCA3 from oligozoospermia or azoospermia patients when compared with controls (Tables 1 and 2). Further analysis of the length of CAG repeats in SCA3, among the 9 oligozoospermia patients carrying a higher number of CAG repeats (>32) in SCA1, did not reveal a higher number of CAG repeats in SCA3 (Table 3). Among these patients, numbers of repeats were between 14 and 33.

Discussion

Our distribution of CAG repeats in SCA1 from healthy males (Fig. 1a) is consistent with a previous finding that normal individuals typically possess 22 to 32 CAG repeats [18]. This study also finds that there are significantly more alleles with expanded CAG repeats in SCA1 of oligozoospermia patients than in the control group (Fig. 1b). More importantly, 3 of the 62 alleles in the oligozoospermia group were shown to have as many as 38 CAG repeats.

This study demonstrates a similar bimodal distribution of CAG repeats in SCA3 in the control group to that of Pan et al and Tsai et al [15, 18], with the two highest peaks at 14 and 26, respectively (Fig. 2a). Pan et al also noted that azoospermia patients have significantly higher frequencies of repeat numbers over 28 [15]. However, this study did not verify their results and showed that neither oligozoospermia

Table 3 CAG repeats in 9 oligozoospermia patients in SCA1 and SCA3 genes

Sample	SCA1	SCA3
o02	29, 38 ^a	14, 27
o06	32, 33	28, 33
o13	32, 33	14, 26
o14	30, 35	26, 27
o23	32, 34	14, 27
o25	28, 33	14, 26
o30	30, 34	14, 14
o31	36, 38	14, 14
o34	29, 38	14, 27

^a CAG repeats that are greater than 32 in SCA1 and 29 in SCA3 are shown in bold type.

nor azoospermia patients carry an SCA3 gene with longer CAG repeats than those of normal individuals (Fig. 2). Further studies using a larger sample are recommended to verify our results.

We further analyzed the SCA3 and the mitochondrial DNA polymerase gamma genes in the oligozoospermia patients with more than 32 CAG repeats in SCA1. CAG repeats are not expanded in SCA3 or mitochondrial DNA polymerase gamma gene in these patients (Table 3 and data not shown). This implies that the upstream regulatory mechanisms leading to the increased CAG repeats in SCA1 are not associated with any defect in global gene regulation.

An increased number of CAG repeats between 39 and 82 in SCA1 has been found to be associated with Spinocerebellar ataxia type 1 [3]. In particular, the anticipation of spinocerebellar ataxia type 1 is known to be high [14]. Children who are born from the ICSI procedure using sperm from men with expanded CAG repeats in SCA1 may have an increased risk of developing this disease, because further increases in the number of CAG repeats may occur during spermatogenesis [14]. This study suggests that there is a potential risk of inheriting type I spinocerebellar ataxia through ICSI from oligospermia patients. We suggest that oligozoospermia patients who plan to undergo ICSI be screened for length of CAG repeats in SCA1. Additional genetic tests in either preimplantation or prenatal stage may be given to reduce the risk of inheriting the disease from an individual with a high number of CAG repeats in SCA1.

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