CAG REPEAT POLYMORPHISM OF THE MITOCHONDRIAL DNA POLYMERASE GAMMA GENE IN MACEDONIAN INFERTILE AND FERTILE MEN

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ABSTRACT

The catalytic subunit of human mitochondrial DNA polymerase gamma (POLG) is encoded by the POLG gene, located on chromosome 15q24 and includes a polymorphic CAG repeat. Analysis of POLG genotypes in some populations has identified an association between the absence of the 10 CAG repeat allele and male infertility and suggested that POLG gene polymorphism should be considered as a possible contributing factor in cases with idiopathic subfertility with normal spermiograms. We undertook to assess whether different POLG CAG alleles are associated with impaired spermatogenesis and infertility/subfertility in Macedonian men. We studied 225 infertile/subfertile men (74 with azoospermia, 56 with severe oligozoospermia, 27 with mild oligozoospermia, 39 with unexplained infertility and normal sperm counts, and 29 with known causes of infertility) and a control group of 123 proven fathers, by fluorescent polymerase chain reaction (PCR) and

analysis on an ABI PRISM™ 310 Genetic Analyzer. The most frequently observed POLG allele was the common one of 10 CAG repeats with a frequency of 87.6% in the infertile/subfertile group and 86.6% in the control group. The homozygous mutant POLG genotype (not10/not10) was found in both groups, 1.6% in the infertile/subfertile patients and 1.8% in the controls. In conclusion, our study showed no association between polymorphism of the POLG gene and infertility in Macedonian men.

Key words: Male infertility; Mitochondrial DNA polymerase gamma (POLG) gene; CAG repeat

INTRODUCTION

Infertility/subfertility affects approximately 5-7% of the male population worldwide and may increase in the future [1]. Infertility/subfertility of undefined cause is referred to as idiopathic infertility/subfertility, but is likely to be of genetic origin. Although the number of candidate spermatogenesis genes is steadily increasing, their pathogenic role is largely unknown. A recent report focused on gene polymorphisms as a cause for impairment of sperm production and male infertility [2].

The mitochondrial DNA polymerase gamma (POLG) gene is located on chromosome 15q24 and contains a polymorphic CAG repeat [3]. The com-

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mon allele contains 10 CAG repeats and occurs at a uniformly high frequency (88-96%) in Eurasian populations [4-6]. The not10 CAG repeat alleles (ranging from six to 15) are considered as mutants. Rovio *et al.* [3] reported a strong association between the absence of the 10 CAG allele and male infertility, specifically with moderate oligozoospermia, and hypothesized that the presence of two mutant alleles would lead to a suboptimal polymerase activity with accumulation of mutations in mtDNA, impaired energy metabolism in the spermatogenic cells and disturbance of sperm production and/or differentiation. Later Jensen *et al.* [7] suggested that the POLG gene polymorphism should be considered as a possible contributing factor in patients with unexplained subfertility and normal semen parameters. However, studies in Italy [8,9] and France [10] did not confirm any influence of the POLG CAG polymorphism on the efficiency of spermatogenesis and male fertility. Recently, a difference has been reported in the number of CAG repeats of the POLG gene between Chinese and European males, and it was proposed that the 10/not 10 CAG genotype may affect sperm motility in Chinese males [11]. The aim of this study was to assess whether different POLG CAG alleles are associated with impaired spermatogenesis and infertility/subfertility in Macedonian men.

MATERIALS AND METHODS

Subjects. A total of 225 male patients attending the Andrology Outpatient Unit at the Clinic for Endocrinology and Metabolic Disorders, Faculty of Medicine, Skopje, R. Macedonia, were enrolled in the study. All gave informed consent to participate in the study. Semen analysis was performed in accordance with World Health Organization (WHO) guidelines [12]. All patients were routinely screened for the presence of sex chromosomal aneuploidies by QF polymerase chain reaction (PCR) analysis and for the presence of Y micro deletions by multiplex PCR analysis of several STS sites in the three AZF regions [13,14]. The group with known causes of infertility included 29 patients; 10 had Y micro deletions, eight were XXY patients, two were XX, one was XYY and eight had obstructive azoospermia. The other 196 patients were divided into four subgroups according to the severity of the spermatogenic defect as determined by semen analysis: azoospermia, $n = 74$; se-

vere oligozoospermia ($<5 \times 10^6/\text{mL}$), $n = 56$; mild oligozoospermia ($>5 \times 10^6/\text{mL}$), $n = 27$; and normozoospermia, $n = 39$. In addition, 123 men who have fathered at least one child by natural conception and whose paternity was proven by DNA analysis, served as controls.

Methods. Genomic DNA was isolated from leukocytes using the Proteinase K/SDS digestion-phenol/ chloroform extraction-ethanol precipitation method [15]. The POLG CAG repeat number was determined by fluorescent PCR amplification of exon 2 using the following primers: direct 5'-ROX-CCA GCT CCG TCC CCG CGT CCG ACC-3' and reverse 5'-GCT GCC CGC CCT CCG AGG ATA GCA C-3'. The size of the PCR product was determined by capillary electrophoresis on an ABI PRISM™ 310 Genetic Analyzer (Applied BioSystems, Foster City, CA, USA) and the size of the PCR products was determined by GeneScan software (Figure 1). The number of CAG repeats predicted by the GenesScan software was compared with the actual CAG repeats determined by direct dideoxy terminator cycle sequencing using a Big Dye Terminator Sequencing Kit v1.0 (Applied BioSystems) in male DNA samples homozygous for 10 and 11 CAG repeats.

Statistical Analysis. Statistical analysis was performed using Chi square and Fisher's exact test. A p value of <0.05 was considered statistically significant for each test.

Figure 1. Electrophoreogram obtained on an ABI PRISM™ 310 Genetic Analyzer (Applied BioSystems) from patients with different POLG CAG genotypes: A) 10/10; B) 10/11; C) 9/11.

RESULTS

Six different alleles that contained from 7 to 12 CAG repeats were detected in the 696 alleles studied (Table 1). The most frequently observed POLG allele was the common allele of 10 CAG repeats with a frequency of 87.6% in the infertile/subfertile group and 86.6% in the control group of fertile males. The frequency of the allele of 10 CAG repeats was similar in all five subgroups of infertile/subfertile patients ranging from 85.1% in the azoospermia group to 90.2% in the severe oligozoospermia group. The second most frequent allele contained 11 CAG repeats, ranging from 8.0% in severe oligozoospermia group to 11.5% in the azoospermia group. The other four POLG CAG alleles were extremely rare.

The frequency of the different POLG CAG genotypes is given in Table 2. The most frequent genotype was the 10/10, 76.9% in the patient group, 74.8% in the control group. This genotype was designated as a homozygous wild type [3]. Five heterozygous genotypes (7/10, 8/10, 9/10, 11/10, 12/10) and two homozygous mutant genotypes (9/11 and 11/11) were

Figure 2. Distribution of POLG CAG genotypes in different groups of infertile/subfertile patients and fertile controls.

also found. The distribution of the 10/10, 10/not10 and not10/not10 genotypes is shown in Figure 2. Homozygous mutant genotypes were found in the control group (two 11/11), azoospermia group (two: one 9/11 and one 11/11), normozoospermia group (one

Table 1. Allele frequency of the POLG CAG repeats in different groups of infertile/subfertile males and fertile controls

Allele	7		8		9		10		11		12		Total
	n	%	n	%	n	%	n	%	n	%	n	%	
Infertile/Subfertile Males (total)	1	0.2	2	0.4	6	1.3	394	87.6	46	10.2	1	0.22	450
• azoospermia					4	2.7	126	85.1	17	11.5	1	0.7	148
• severe oligozoospermia	1	0.9			1	0.9	101	90.2	9	8.0			112
• mild oligozoospermia			1	1.8			48	88.9	5	9.3			54
• normozoospermia			1	1.3			68	87.2	9	11.4			78
• known cause of infertility					1	1.7	51	87.9	6	10.3			88
Fertile Controls	1	0.4	2	0.8	1	0.4	213	86.6	26	10.6	3	1.2	246

Table 2. Genotype frequency of the POLG CAG repeats in different groups of infertile/subfertile males and fertile controls

Genotype	10/10		7/10		8/10		9/10		10/11		12/12		9/11		11/11		Total
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Infertile/Subfertile Males (total)	173	76.9	1	0.4	2	0.9	5	2.3	39	17.4	1	0.4	1	0.4	3	1.3	225
• azoospermia	54	73.0					3	4.0	14	18.8	1	1.4	1	1.4	1	1.4	74
• severe oligozoospermia	45	80.4	1	1.8			1	1.8	9	16.1							56
• mild oligozoospermia	21	77.8			1	3.7			5	18.5							27
• normozoospermia	30	76.9			1	2.6			7	17.9					1	2.6	39
• known cause of infertility	23	79.3					1	3.5	4	13.7					1	3.5	29
Fertile Controls	92	74.8	1	0.8	2	1.6	1	0.8	22	17.9	3	2.4			2	1.6	123

11/11) and the known cause group (one 11/11) (Table 2 and Figure 2). No homozygous mutant genotypes were found in the severe and mild oligozoospermia groups. The relatively higher frequency of the homozygous mutant genotypes in these three groups (2.8, 2.6 and 3.5%) in comparison with the control group (1.6%) was not statistically different.

DISCUSSION

Whereas two related studies have indicated a relationship between the presence of the not10/not10 genotype and oligo(astheno)zoospermia [3] and unexplained subfertility [7], we have found the homozygous mutant genotype to have a similar frequency in infertile/subfertile patients and fertile controls. We observed no significant difference in the distribution of the three POLG genotypes (homozygous wild, heterozygous and homozygous mutant) in our five subgroups of patients (azoospermia, severe oligozoospermia, mild oligozoospermia, normozoospermia and known cause of infertility). These results agree with those of three other studies [8-10], and suggest that the POLG CAG polymorphism does not play a significant role in male infertility/subfertility. Similarly *in vitro* studies have shown that deletion of the CAG repeat has no detectable effect on mitochondrial function [16]. A recent meta-analysis presenting a summary of the polymorphisms that have been associated with male fertility, also revealed no significant association between POLG polymorphism and male fertility [2]. In conclusion, we have found no association between polymorphism of the POLG gene and infertility in Macedonian men.

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