INTRODUCTION

Infertility is a major health problem today, affecting about 15.0% of couples trying to have a child. Impaired fertility of the male is causative in 20.0% of infertile couples and contributory in up to another 30.0-40.0%. Infertility already affects about 5.0-7.0% of the general male population and may further increase in the future, considering the apparent trend of declining sperm count in industrialized countries. Despite enormous progress in the understanding of human reproductive physiology, the underlying cause of male infertility remains undefined in about 50.0% of cases, which are referred to as idiopathic infertility [1]. Most idiopathic cases are likely to be of genetic origin because the number of genes involved in human spermatogenesis is probably over 1 thousands. At present, only a few of the genes implicated in the processes of testis determination, testis descent and spermatogenesis have routine clinical importance. These include the cystic fibrosis transmembrane conductance regulator (CFTR) gene, whose mutations cause cystic fibrosis and absence of vas deferens and the androgen receptor (AR) gene, whose mutations cause the androgen insensitivity syndrome and spermatogenic damage.

Common Genetic Causes of Male Infertility. Chromosomal anomalies and microdeletions of the azoospermia factor (AZF) regions of the Y chromosome are the only commonly known genetic causes of spermatogenic failure. The frequency of these two genetic anomalies increases with the severity of the spermatogenic defect, reaching up to an overall 30.0% (15.0% karyotype abnormalities and 15.0% of AZF microdeletions) in azoospermic men.

Sex chromosome aneuploidies, such as 47,XXX (Klinefelter’s syndrome), 47,XYY and 46,XX males are the most common chromosome anomalies occurring at birth and in the population of infertile males [2]. Klinefelter’s syndrome is a form of primary testicular failure with a high prevalence in infertile men, up to 5.0% in severe oligozoospermia and 10.0% in azoospermia.

Y chromosome microdeletions represent the etiological factor of 10.0-15.0% of idiopathic azoospermia and severe oligozoospermia [3]. The frequency of AZF deletions in infertile men ranges from 5.0 to 20.0% in worldwide surveys [4]. Y chromosome microdeletions are found almost exclusively in patients with azoospermia or severe oligozoospermia [5]. The prevalence of Y chromosome microdeletions in the infertile males from the Republic of Macedonia is 6.4%, in patients with azoospermia 16.7% and 2.8% in those with severe oligozoospermia.
Deletions most frequently involve the AZFc region, less frequently the AZFb region, and only rarely the AZFa region. The most frequent deletions in Macedonian males are AZFc deletions, while AZFa deletions have not been detected [7,8].

Partial deletions within the AZFc region (gr/gr and b2/b3) that remove smaller portions of the AZFc region (1.6 and 1.8 Mb) are much more common and are present at various frequencies in different Y chromosome haplogroups [9]. While the association of the complete AZFc deletion with spermatogenic failure is well established, the role of partial AZFc deletions on spermatogenesis and male infertility is still controversial.

In addition to deletions, different duplications at the AZFc region have been reported. Duplications can occur on a chromosome with a partial AZFc deletion and generate a chromosome with four DAZ genes, but lacking some sequence tagged site (STS) markers [10,11]. Recently, an AZFc partial duplication has been shown to be a risk factor for male infertility in Taiwan [12].

Gene Polymorphisms and Male Infertility. Analysis of Y chromosome haplogroups, defined by single nucleotide polymorphisms (SNPs), has become a standard approach for studying the origin of human populations and measuring the variability among them. A few groups have studied the possible association of Y chromosome haplogroups with male infertility and Y chromosome microdeletions, but conflicting results have been published. Some recent studies suggested that a Y chromosome background is an important factor that affects partial AZFc deletion formation and its contribution to spermatogenic failure [14].

We have used a hierarchical analysis of 28 SNP markers by multiplex PCR followed by single base extension reactions using a multiplex SNapshot kit to determine the Y chromosome haplogroups in men from our country [15]. Our initial results showed slight differences in the distribution of the Y chromosome haplogroups such as higher frequency of the R1a haplogroup in infertile patients with a milder phenotype in comparison with those with azoospermia and severe oligozoospermia and fertile controls.

We have studied in detail the Y chromosomal background of different Y chromosome deletions detected in men from our country. Several different Y chromosome haplogroups were determined in men with complete AZFc (b2/b4) deletions and gr/gr deletions. All infertile males with b2/b3 deletion belong to the Hgr E3b1 anomaly, while the only fertile man with this deletion falls within the Hgr N3 anomaly. Most of the men with the b2/b4 duplication, both infertile and fertile, were identified as Hgr R1a, but the frequency of this Hgr was higher in infertile men. There was also a difference in the distribution of the Y chromosome haplogroups in males with the b2/b3 duplication.

The analysis of polymorphisms in genes involved in spermatogenesis represents one of the most exciting areas of research in genetics of male infertility [16]. Polymorphisms in these genes are considered potential risk factors that may contribute to the severity of spermatogenic failure. Polymorphisms in different genes [CAG repeats in AR and DNA polymerase γ (POLG) genes, C677T mutation in 5-methyltetrahydrofolate reductase (MTHFR), A260G and A386G in the DAZL gene, different polymorphisms in FSHR, ERα, protamine...
1 and 2, etc.] have been studied for possible association with male infertility but many of them have presented contradictory results. It is likely that only polymorphisms in association with a specific genetic background and/or with environmental factors can lead to spermatogenic impairment. We have also studied the possible association of several different polymorphisms with male infertility. There was no association between the POLG polymorphism and infertility in Macedonian men [17]. We found a significantly higher percentage of long CAG repeats in patients with mild oligozoospermia indicating the possible association of CAG repeat numbers in exon 1 of the AR gene and mild oligozoospermia [18]. Our preliminary results suggest that there is no association between the MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A666G polymorphisms and male infertility. The first study that investigated CNVs in patients with severe oligozoospermia and Sertoli cell only syndrome (SCOS) was published only recently [20]. This study provided a number of candidate genes, possibly causing or being risk factors for spermatogenic failure. Using array CGH analysis we have also identified several CNVs (UGT2B17 gene on chr4 q13.2; STEAP2 gene on chr7 q21.13; TPTE gene on chr21 p11.2-11.1 and H2BFWT on chrX q22.2) that might be associated with impaired spermatogenesis and male infertility.

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