

Proceedings of the MACPROGEN Final Conference held  
at Ohrid, Republic of Macedonia, March 29-April 1 2012

## GENETIC CAUSES OF MALE INFERTILITY

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### INTRODUCTION

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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,XXY (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 oligozoosper-

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mia [6]. 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].

**Screening for Common Genetic Causes of Male Infertility by Quantitative Fluorescent-Polymerase Chain Reaction.** Screening for chromosomal abnormalities is usually done by cytogenetic analysis and for AZF deletions by polymerase chain reaction (PCR) analysis of several STSs in the three AZF regions. Recently, we described a multiplex quantitative fluorescent (QF)-PCR method that allows simultaneous detection of the most common genetic causes of male infertility, *i.e.*, sex chromosomal aneuploidies and AZFc and AZFb deletions, and some potential risk factors such as partial AZFc deletions/duplications and *AR* CAG repeats [8]. This multiplex QF-PCR analysis was shown to be a rapid, simple, reliable and inexpensive method that can be used as a first-step genetic analysis in infertile patients. Recently, we presented a modified system, where we have included additional markers in the AZFa and AZFb region, as well as a marker for determination of the X/chromosome 3 ratio [13].

Our results showed that Klinefelter's syndrome and complete AZFc deletions are the most common genetic causes of azoospermia. Partial AZFc deletions as well as AZFc duplications were present in both infertile and fertile men. They may represent a risk factor for male infertility when present on certain Y chromosomal backgrounds.

### **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  $\gamma$  (*POLG*) genes, C677T mutation in 5-methylenetetrahydrofolate reductase (*MTHFR*), A260G and A386G in the *DAZL* gene, different polymorphisms in *FSHR*, *ER $\alpha$* , 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* A66G polymorphisms and male infertility. Of the nine SNPs evaluated in eight different genes (*FASLG*, *JMJDIA*, *LOC203413*, *TEX15*, *BRDT*, *OR2W3*, *INSR* and *TAS2R38*), we found significant association for three SNPs (rs5911500 in the *LOC203413*, rs3088232 in the *BRDT* and rs11204546 in the *OR2W3* genes, respectively) [19].

Copy number variations (CNVs) represent an important source of genetic diversity with remarkable differences between individuals. Copy number variations can cause spermatogenic failure by their increased number or specific distribution that could result in defective recombination, meiotic failure and loss of germ cells. Copy number variations might also affect the activity of genes important for spermatogenesis. 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.

## ACKNOWLEDGMENTS

This study was supported by grant CRP/MAC09-01 from the International Centre for Genetics Engineering and Biotechnology, Trieste,

Italy (to D. Plaseska-Karanfilska). We are grateful to Dr. Rafael Oliva for the training of Ms. Sanja Kiprijanovska in the genomic and proteomic investigation of spermatozoon that she received during her stay at the University of Barcelona, Faculty of Medicine, Department of Physiological Sciences, Human Genetics Laboratory, Barcelona, Spain. Ms. Kiprijanovska's 2-week stay was supported by the FP7 project No. 229458 from the European Commission.

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