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The association of gr/gr deletion in the Y chromosome and impaired spermatogenesis in Bulgarian males: a pilot study

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Abstract

Background: The microdeletions of the Y chromosome are associated with a decreased number of sperm cells in the ejaculate and male infertility. One such deletion is the gr/gr, which leads to reduction of the alleles in the DAZ gene. In order to evaluate its role, 30 patients with sperm count below $5 \times 10^6/\text{mL}$ and 30 normal fertile men were genotyped for microdeletions in Azoospermia Factor (AZF) region, including gr/gr deletion.

Results: Twenty-one men (70.00%) from the males with impaired fertility had a normal genotype, seven men (23.33%) were carriers of a gr/gr deletion, and two men (6.67%) demonstrated a deletion of the AZFc region. From the control group, there were 22 men (73.33%) with no deletion, seven (23.33%) carried a gr/gr deletion, and one man (3.33%) carried a possible deletion in the b1-b4 region of the Y chromosome. The gr/gr deletion of the Y chromosome showed a statistically nonsignificant difference ($p = 0.94$, $\alpha < 0.05$) with the control group (chi-square, degrees of freedom 0.006).

Conclusion: The results from the research show no association between this deletion and male infertility in the studied population. Despite the small sample size, the high frequency of the gr/gr deletion, found in the control group, suggests that this variant may not be associated with impaired spermatogenesis.

Keywords: Male, Infertility, gr/gr, deletion, Y chromosome

Background

According to the World Health Organization, infertility is defined as a disease of the reproductive system described by the inability to conceive a child after 1 year or more of regular unprotected sexual intercourse [26]. This is a global issue, affecting around 15% of the couples trying to conceive [1, 10]. Male infertility solely is responsible for 20–30% of the reproductive problems of the couples and over the last few years is gaining more clinical attention [1].

After Klinefelter syndrome the microdeletions of the azoospermia factor (AZF) region in the long arm of the

Y chromosome are the second most common reason for male infertility [11]. In this region, there are three spermatogenesis loci, named AZFa, AZFb, and AZFc [23].

AZFc deletions are the most common type. In most of the cases, they involve regions b2/b4, b1/b3, b2/b3, and gr/gr, which leads to the lack of DAZ gene [12, 19].

The last one, the gr/gr deletion, was first noted as a risk factor for impaired sperm production in 2003, because it removes a proximal part of the AZFc region, which is important for the normal spermatogenesis [16]. The deletion is characterized by the lack of sY1191 marker and the presence of sY1291 marker [16]. However, the deletion does not remove all genes, found in the AZFc locus, but reduces the number of the DAZ genes by removing two of them [16].

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Since 2003, there were studies in different countries about the potential impact of the *gr/gr* deletion on the spermatogenesis [5–8, 13, 16, 18]. However, their results are contradictory and that is why this deletion remains controversial.

The aim of this research was to analyze the role of the *gr/gr* deletion in Bulgarian males with oligo- or azoospermia and to report data, since there is no information for our country.

Methods

A total of 60 samples divided into two groups were analyzed in the Laboratory of Medical Genetics in Varna, Bulgaria. The first group consisted of 30 patients with sperm count below $5 \times 10^6/\text{mL}$. The second group included 30 normal fertile men without previous history of reproductive problems. All of the case subjects were affected by unexplained primary infertility and were without anatomical, microbial, viral, or endocrine diseases. Patients, who had mumps-related orchitis, cryptorchidism, varicocele, or other conditions, which could explain the low sperm count, were excluded from the study. All of the participants signed an informed consent before the analysis, and the study was approved by the ethics committee of Medical University Varna.

Whole blood samples were used to extract DNA and search for the presence or absence of AZFa, AZFb, AZFc, and *gr/gr* deletions. AZF System Y Chromosome (Sacace Biotechnologies, Italy) was used for the detection of Y microdeletions as described by the manufacturer. *Gr/gr* deletion was diagnosed by genotyping sY1291 and sY1191 using multiplex real-time polymerase chain reaction (PCR) (QuantStudio Dx, Applied Biosystems, USA) according to the protocol described by A. Shaqalaih et al [17]. PCR was performed in 20 μL reaction, containing 5 \times HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Estonia), primer mix, and patient's DNA.

The absence of any marker was confirmed by performing 2% agarose gel electrophoresis for the detection of

ethidium bromide-stained products and singleplex polymerase chain reaction (Fig. 1). The *gr/gr* deletion was characterized by the lack of sY1191 marker and the presence of sY1291 marker.

Differences between the two groups were analyzed by GraphPad Prism software (version 8) using chi-square and Fisher tests. A difference was considered significant at a p value < 0.05 .

Results

The median age of the men with primary infertility was 34.27 years (minimum, 22.00; 25% percentile—30.00; 75% percentile—39.25; maximum 47.00). The median age in the control group was 34.00 years (minimum, 27.00; 25% percentile—31.00; 75% percentile—37.50; maximum, 48.00). Nine of the case subjects (30.00%) showed azoospermia and 21 (70.00%) oligozoospermia with a sperm count varying between 1×10^6 and $5 \times 10^6/\text{mL}$.

None of the participants from both groups had an AZFa or AZFb deletion. Two men from the patients group had an AZFc deletion. None of the control subjects demonstrated an AZFc deletion.

A total of 21 men (70.00%) from the case group showed normal genotype for the *gr/gr* deletion, seven men (23.33%) carried an isolated *gr/gr* deletion, and two men (6.67%) showed no amplification of both markers and were later diagnosed with deletion of the whole AZFc region (Fig. 2).

The frequencies for the control group were as follows: 21 men (73.33%) had no deletion, seven (23.33%) did not express the sY1291 marker and carried a *gr/gr* deletion, and one man (3.33%) showed no amplification only of the sY1191, indicating a possible deletion in the b1-b4 region (Fig. 2). However, other markers should be further investigated to confirm this type of deletion.

The studied polymorphism of the Y chromosome showed a statistically nonsignificant difference ($p = 0.94$, $\alpha < 0.05$) with the control group (chi-square, degrees

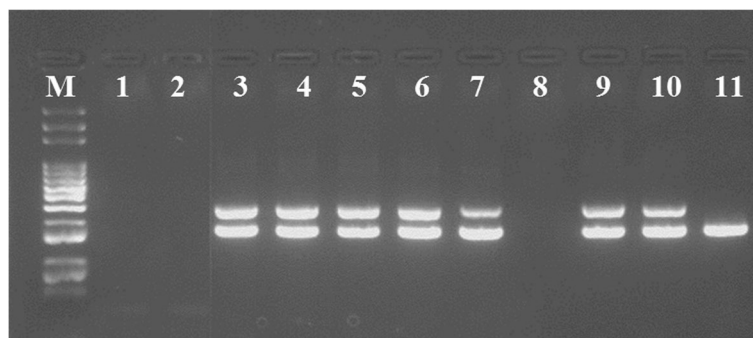


Fig. 1 Gel electrophoresis for detection of *gr/gr* deletion. Lane 1, no template control; lane 2, female patient; lanes 3–7, 9, and 10, normal genotype; lane 8, deletion of both markers; lane 11, no sY1291 marker detected, which indicates a *gr/gr* deletion; M, 1 kb DNA ladder

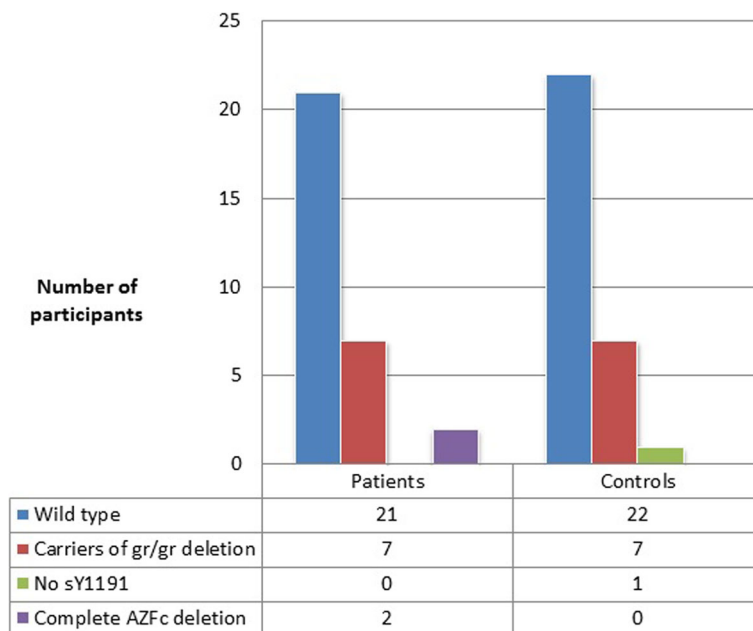


Fig. 2 Prevalence distribution of the genotypes of the participants for the gr/gr deletion

of freedom (*df*) 0.006). The polymorphism was not distributed differently in the studied groups and was not associated with impaired spermatogenesis.

In order to estimate if the gr/gr deletion had aggravating effect on the spermatogenesis in the group of patients with primary infertility, we performed Fisher's exact test. However, there was no statistical difference ($p = 0.11$, $\alpha < 0.05$) between patients with oligo- and azoospermia.

Discussion

The AZFc deletions are the most common type and depending on the missing genes, they could result into impaired spermatogenesis [24]. The genes in this locus are organized in a palindromic sequence, DAZ1/2 and DAZ3/4. The high prevalence of deletions occurring in AZFc could be explained by its specific structure, which could lead to intrachromosomal aberrations during non-allelic homologous recombination and high percentage of deletions of genes, located in the AZFc locus [19].

According to several research groups, the gr/gr deletion is a potential risk factor for a low number of sperm cells, found in the ejaculate [6, 7, 13]. However, there are also studies which deny its role and find no correlation between the deletion and impaired spermatogenesis [5, 25].

Despite the studies carried over the last couple of years in different populations, the impact of the gr/gr deletion on the spermatogenesis remains uncertain. As a result of this, testing for this marker is not recommended in the last guideline of the European Academy of Urology [11].

The findings from our study are in agreement with similar experiments from other countries, where there was no statistical difference in the distribution of gr/gr deletion among case and control subjects [2, 8, 21]. It is also suggested that no matter the lack of expression for sY1291 and the presence of gr/gr deletion, all copies of the DAZ gene are present. This could be due to a duplication of these genes, which occurred after the deletion as a compensatory mechanism [14]. It is a possible explanation of the fact that gr/gr deletions can be found in fertile patients with normozoospermia [22].

Our data showed no aggravating effect of the presence of gr/gr deletion on the impaired spermatogenesis after comparing patients with azoospermia and oligozoospermia. However, more patients with azoospermia should be included in order to further prove this finding.

Two of our case subject tested negative for both sY1291 and sY1191 markers and were later diagnosed with a complete deletion of the AZFc region. It could be suggested that only one missing marker is not specific enough for a deletion of the Y chromosome and only when both are absent this could result in an impaired spermatogenesis due to Y microdeletion.

Nevertheless, our results are in contradiction to the conclusions from two meta-analyses [4, 20]. The reason for this could be differences between the included case and control patients or due to different ethnic background since the Y chromosomal haplogroup could play a role in the frequency of gr/gr deletion. This deletion could be a polymorphic variant and because of the controversial results from different studies is not

recommended to include it in the test panel for Y microdeletions [11].

One possible limitation of the study is the small number of included patients. Despite of that, there is a strong tendency towards nonstatistical significance since the p value is close to 1.00. That is why regardless of the small number of participants, the study is not underpowered.

Moreover, the prevalence and clinical effect of the deletion may depend on the Y chromosome background since the *gr/gr* deletion is a common finding among D2b, Q3, and Q1 Y haplogroups found in Asia. In men from Japan and China, who carry this mutation, there was no impaired spermatogenesis and it was present even in normozoospermic men [18, 25].

However, in Bulgaria, different Y haplogroups are found. Around 40% of them belong to haplogroups E and I, 20% to R, and another 20% to J and G haplogroups. The rest 20% are divided among other haplogroups, which occur at a very low frequency [9]. There is data suggesting that partial deletions of the Y chromosome are a common finding in men from E haplogroups [3, 15], but we did not test our patients for their Y chromosomal background. It is unknown whether the Y haplogroups, found in Bulgaria, predispose to deletions and could explain the high prevalence of the *gr/gr* deletion in our study.

Conclusions

The results from the presented research show no association between the *gr/gr* deletion and male infertility in the studied population. One limitation is that the sample size is small with limited power and should be increased. Due to the high frequency of the *gr/gr* deletion, found in the control group, the impact of this mutation on the spermatogenesis is uncertain. However, more research is needed to confirm its effect on male infertility.

Abbreviations

AZF: Azoospermia factor; PCR: Polymerase chain reaction; Df: Degrees of freedom

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Not applicable

Authors' contributions

ML conducted the analysis, drafted the work, and did the data analysis. TC made substantial contributions to the design of the work and interpretation of the data. LA supervised the work process and revised the manuscript. All authors have read and approved the submitted manuscript. All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated and resolved and the resolution documented in the literature. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All of the participants signed an informed consent before the analysis, and the study was approved by the ethics committee of Medical University Varna, No. 75. The obtained consent was written. Since none of the participants was minor, there was no need for parental consent.

Consent for publication

Not applicable since no personal information was used.

Competing interests

The authors declare that they have no competing interests.

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