

Original Article

Lack of Association Between Partial Y chromosome AZFc-gr/gr Deletions and Male Infertility

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Abstract

A microdeletion in the azoospermia factor (AZF) region of the human Y chromosome long arm is the second most common genetic factor causing male infertility and spermatogenetic failure. The current study aimed to evaluate the occurrence of AZFc-partial gr/gr deletions in a sample of 260 infertile azoospermia and 40 fertile men as a control group from the Kurdish community using the gel-based PCR technique. To understand whether these deletions were associated with their fertility problems. Two pairs of gr/gr primers (sY1291 and sY1191) and a primer of Hetero-chromatin sY160-F were used for screening for gr/gr deletion in a single PCR reaction according to EAA and EMQN protocol. Based on the results of this study, no AZF/c gr/gr deletions were found in either the infertile or fertile participants. Therefore, this finding confirms the concept that these AZFc gr/gr partial deletions are improbable to have an important impact on infertility, and the hereditary risk associated with these partial deletions is much reduced compared to deletions that affect the entire AZFc region.

Keywords: Male Infertility, Y chromosome, Azoospermia, AZFc-gr/gr deletion, PCR technique.

INTRODUCTION

Infertility is a problem of the reproductive system, and can be identified by an inability of couple to get pregnant after at least a year of normal, unprotected sexual activity. Infertility is a worldwide problem, impacting approximately 15% of couples attempting to conceive and around 50% of these conditions are caused by problems with men. Male infertility, which is generally described as a male's inability to conceive in a healthy, fertile female, accounts for 20–30% of all cases of infertility. The WHO's statistics data show that around 48 million couples and 186 million people worldwide have infertility¹. Male infertility is mostly caused by spermatogenic diseases, which are increasingly understood to have a genetic basis. The high frequency of segmental duplications that underlie the wide variety of deletions and duplications seen in different sites on the human Y chromosome make it genetically dynamic and prone to significant change. Since the Yq locus has an array of genes that are transcribed in the testis and governs a specific role in spermatogenesis, eventually infertility would result from losing these

regions²⁻⁴. The male-specific region of the Y chromosome (MSY), which makes up 95% of the chromosome's length, contains the areas that define maleness in humans and the genes required for spermatogenesis⁵. The azoospermia factor (AZF) regions are located on the long arm of the Y-chromosome within the MSY region, which contains genes necessary for spermatogenesis⁶.

According to various research studies 10% of men with azoospermia have microdeletions in the three types of azoospermia factor, AZFa (the most proximal segment), AZFb (the middle), and AZFc (distal). These deletions eliminate several genes that are necessary for the development and maintenance of male germ cells (spermatogenesis) in the testicles⁷. Because the AZFc area contains repeating sequences and palindromes, it is particularly sensitive to deletions and the entire deletion of AZFc involves the b2/b4 region which includes 12 genes and transcriptional units in numerous copy numbers. In addition to b2/b4, the AZFc locus contains a number of partial deletions, including b1/b3 (1.6 Mb), b2/b3 (1.8 Mb), and gr/gr

(1.6 Mb). These deletions eliminate distinct parts of the AZFc, but the genes that are deleted are nearly identical⁸. Due to its high deletion frequency and the presence of multicopy genes linked to male infertility, the AZFc region, which covers about 4.5 Mb of the distal part of the Yq, is one of the most thoroughly studied AZF loci⁷.

The gr/gr deletion results in the loss of two of the four DAZ gene copies, one of the two CDY1 and BPY2 gene copies along with one of the three copies of BPY2 and two of the four DAZ genes. The term 'gr/gr' deletion, named for the fluorescent probes (green and red), is used when it was first characterized⁸. Deletion or removing half of the AZFc region is recognized by partial gr/gr deletion. This deletion is distinguished by the absence of the sY1191 marker and the existence of the sY1291 marker. The deletion, however, does not remove all of the genes found in the AZFc locus, but it does reduce the number of DAZ genes by removing two of them⁸. Since 2003, researchers in various countries have been investigating the potential impact of the gr/gr deletion on spermatogenesis but their findings remain controversial^{9,10}.

This study aimed to determine whether infertile Kurdish patients possessed partial AZFc gr/gr deletions and whether these deletions were associated with their reproductive problems. To enhance perception regarding the genetic determinants that contribute to male infertility within the Kurdish population, with the potential of providing improved diagnostic and therapeutic alternatives for the suffering.

MATERIALS and METHODS

Patients and Control

The present investigation has been authorized by the Research Ethics Committee at the College of Science, Salahaddin University-Erbil (SUE) Iraq, No. 4S/345, 24/12/2019, and informed permission has been obtained from every one of the attendees. This research was carried out on 260 Iraqi Kurdish infertile Patients in Erbil province. All cases have been identified with azoospermia according to WHO guidelines¹¹, with median age 35 year. About 40 healthy males with

paternity evidence made up the control group with a median age of 36 years.

II. Molecular Methods:

A. Genomic DNA extraction

All participants gave permission to the drawing of two milliliters of venous blood using a sterile syringe, and blood samples were collected in anticoagulant EDTA tubes. Blood samples were used to extract genomic DNA in accordance with the instructions provided by the manufacturer (Genomic DNA Mini Kit, Geneaid, Taiwan). The process steps have been carried out in lines with a manufacturing protocol. The NanodropTM 1000 spectrophotometer (Thermo Scientific, USA) was utilized to evaluate each DNA sample's quality, quantity, and purity.

B. PCR testing for Y chromosome AZFc-partial gr/gr deletion

For each patient and control, molecular screening was conducted according to the EAA and EMQN protocol for analysis of AZFc-partial gr/gr deletion¹². Two pairs of gr/gr primers (sY1291 and sY1191) and a primer of Hetero-chromatin sY160-F (Table 1) were used for screening for gr/gr deletion through gel based PCR reaction. The PCR condition program is shown in the (Table 2).

C. Primer preparation

Sterile D.W. was added to each microcentrifuge vials contained lyophilized specific primer to obtain a concentration of 100 pmol / μ l of each primer according to manufactures protocol (Macrogen; LIGO).

D. PCR sample preparation

The PCR mixture was made up with a final volume of 25 μ l and it consisted of 12.5 μ l of 2X Master Mix (AMPLIQON), 1 μ l of each primer with a concentration of 10 pM (Macrogen; LIGO), and 5 μ l of genomic DNA sample as a template. The mixture was then completed to the 25 μ l volume with deionized distilled water. The amplification PCR conditions was as follows: initial denaturation for 5 min at 95°C; 35 cycles, denaturation 30 s at 94°C; annealing 30 s at 60°C; and elongation 30s at 72°C and a final elongation step of 10 min at 72°C by using the thermal cycler machine (Alpha thermal Cycler; code: AC196).

Table 1. The table shows the locus of types AZFc-partial gr/gr, primer name and primer sequence, PCR product size and status in case of deletions

Locus	Primer	Sequence	Product size (bp)	Deletion status
gr/gr sY1291	sY1291F	5'-TAA AAG GCA GAA CTG CCA GG-3'	527	Absent
	sY1291R	5'-GGG AGA AAA GTT CTG CAA CG-3'		
gr/gr sY1191	sY1191F	5'-CCA GAC GTT CTA CCC TTT CG-3'	385	Present
	sY1191R	5'-GAG CCG AGA TCC AGT TAC CA-3'		
Heterochro matin sY160	sY160	5'-TAC GGG TCT CGA ATG GAA TA-3'	236	Present
	sY160	5'-TCA TTG CAT TCC TTT CCA TT-3'		

Table 2. The thermalcycler conditions program

Step	Temperature	Time	cycles
Initial denaturation	95 °C	5min	1
Denaturation	94 °C	30 sec	35
Annealing	56 °C	30 sec	
Extension	72°C	45 sec	
Final extension	72°C	10 min	1
Storage	10°C		

E. Gel electrophoresis

The amplified PCR product (5µl) for each sample was separated on a 1.5% agarose gel (containing 0.5 microliters of ethidium bromide) with 40-50 minutes electrophoresis duration¹³. The electrophoresed gel was analyzed under UV light using the gel documentation system (Proxima 2500 Isogene Life science, Netherlands). A 100bp DNA ladder was used to evaluate the PCR outcome and confirm the size of each amplicon (GeneDirex).

RESULTS

In this study, 260 infertile Kurdish males and 40 fertile males served as a control group were screened for AZFc-gr/gr partial deletion from their Y chromosomes by gel-based PCR technique. No AZF/c gr/gr deletions were detected in either the infertile or fertile groups. According to the results of this study all participants has regions of AZF/c gr/gr markers (Figure 1, 2 and 3).

DISCUSSION

This study is the first study that investigated the partial AZFc gr/gr deletions in infertile males among the Kurdish population. According to the results of this study there is no association between the AZF/c gr/gr deletion and infertility. Despite research conducted in several populations over the

last few years, the impact of the gr/gr deletion on spermatogenesis remains unknown and controversial. As a result of this, the most recent guideline from the European Academy of Urology does not recommend testing for this marker¹⁴.

Several research groups indicate that the gr/gr deletion is a risk factor for a low quantity of sperm cells in the ejaculate^{15,16}. Some studies have shown a significant association between partial AZFc deletions and spermatogenic failures^{8,15,17,18}. However, there are also studies which deny its role and find no correlation between the partial AZFc deletion and impaired spermatogenesis^{10,19,20}.

However, because the genetic risk of these partial deletions is much lower than that of deletions affecting the full AZFc, these partial deletions are unlikely to have a significant impact on spermatogenesis. The data on partial deletions of the AZFc region is currently scarce, however, it is estimated that 4% to 6% of males with spermatogenetic failure have partial deletions. Furthermore, certain patterns of deletion are more frequent in other populations, such as Eastern Siberian Yakuts, and are compatible with normal spermatogenesis and fertility^{21,22}. These studies suggest that geographical and ethnic differences might influence the frequencies of AZF deletions

and partial deletions of the AZFc region, as well as the deletion patterns and, possibly, the phenotypic expression. In addition, there is an overall belief that the fertility potential of an older man is fairly

well conserved. However, the evidence supports the concept that increased paternal age is correlated with an increase in sperm DNA double-strand breaks²³.

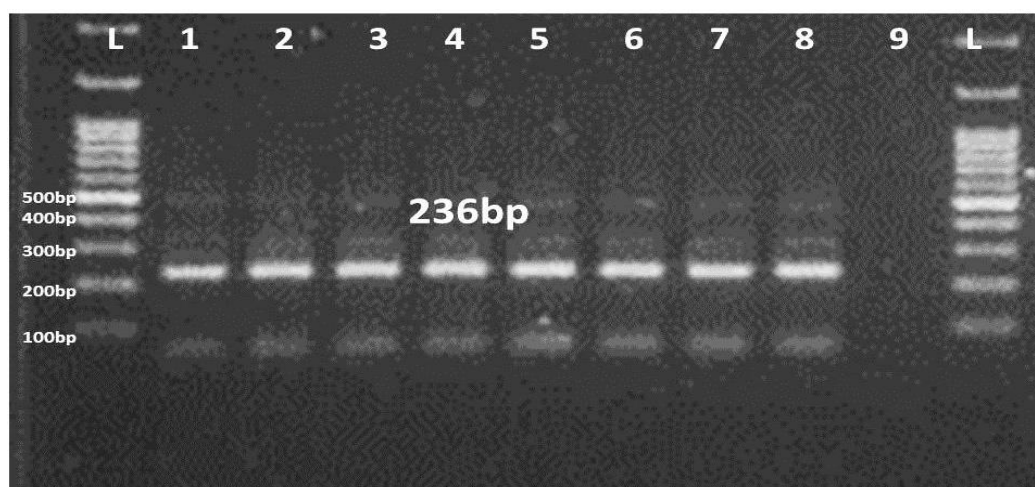


Figure 1. 1X TBE 1.5% agarose gel electrophoresis of PCR result of infertile and fertile male with: Lane L represent 100bp size DNA ladder marker, Lane 1-8 represent result PCR mixture of Hetero-chromatin (sY160), Lane 9 represents negative control.

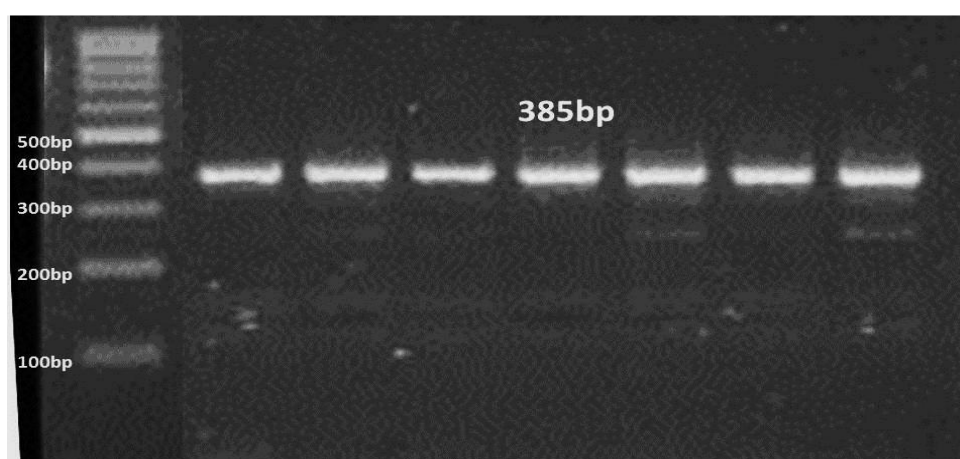


Figure 2. 1X TBE 1.5% agarose gel electrophoresis of PCR result of infertile and fertile male: Lane L represent 100bp size DNA ladder marker, Lane 1-7 represent result PCR mixture of gr/gr (sY1191).

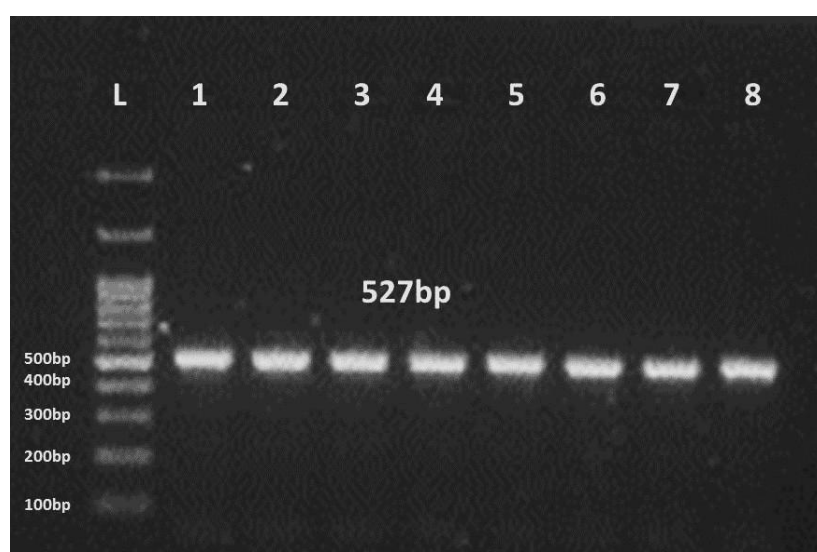


Figure 3. 1X TBE 1.5% agarose gel electrophoresis of PCR result of infertile and fertile male: Lane L represent 100bp size DNA ladder marker. Lane 1-8 represent result PCR mixture of gr/gr (sY1291)

CONCLUSIONS

According to the findings of this study, no partial AZFc-gr/gr deletions were detected in infertile and fertile Kurdish males, suggesting no association between the AZF/c-gr/gr deletion and infertility.

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Conflict of Interest

The authors declare they have no conflicting interests.

REFERENCES

- Akalewold M, Yohannes GW, Abdo ZA, Hailu Y, Negesse A. Magnitude of infertility and associated factors among women attending selected public hospitals in Addis Ababa, Ethiopia: a cross-sectional study. *BMC Women's Health*. 2022/01/11 2022;22(1):11. doi:10.1186/s12905-022-01601-8
- Jungwirth A, Diemer T, Dohle G, et al. EAU guidelines on male infertility. *Eur Urol*. 2013;7:226-241.
- Vander Borgh M, Wyns C. Fertility and infertility: Definition and epidemiology. *Clin Biochem*. Dec 2018;62:2-10. doi:10.1016/j.clinbiochem.2018.03.012
- Hallast P, Ebert P, Loftus M, et al. Assembly of 43 human Y chromosomes reveals extensive complexity and variation. *Nature*. Sep 2023;621(7978):355-364. doi:10.1038/s41586-023-06425-6
- Rhie A, Nurk S, Cechova M, et al. The complete sequence of a human Y chromosome. *Nature*. Sep 2023;621(7978):344-354. doi:10.1038/s41586-023-06457-y
- Hotaling J, Carrell DT. Clinical genetic testing for male factor infertility: current applications and future directions. *Andrology*. May 2014;2(3):339-50. doi:10.1111/j.2047-2927.2014.00200.x
- Witherspoon L, Dergham A, Flannigan R. Y-microdeletions: a review of the genetic basis for this common cause of male infertility. *Translational Andrology and Urology*. 2021;10(3):1383.
- Repping S, Skaletsky H, Lange J, et al. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *The American Journal of Human Genetics*. 2002;71(4):906-922.
- Lu VM, O'Connor KP, Shah AH, et al. The prognostic significance of CDKN2A homozygous deletion in IDH-mutant lower-grade glioma and glioblastoma: a systematic review of the contemporary literature. *J Neurooncol*. Jun 2020;148(2):221-229. doi:10.1007/s11060-020-03528-2
- Levkova M, Chervenkov T, Angelova L. The association of gr/gr deletion in the Y chromosome and impaired spermatogenesis in Bulgarian males: a pilot study. *Middle East Fertility Society Journal*. 2020;25(1):1-5.
- Organization WH. *WHO laboratory manual for the examination and processing of human semen*. World Health Organization; 2021.
- Krausz C, Hoefsloot L, Simoni M, Tuttelmann F, European Academy of A, European Molecular Genetics Quality N. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. *Andrology*. Jan 2014;2(1):5-19. doi:10.1111/j.2047-2927.2013.00173.x
- Brown TA. *Gene cloning and DNA analysis: an introduction*. John Wiley & Sons; 2020.
- Krausz C, Hoefsloot L, Simoni M. European Academy of Andrology; European Molecular Genetics Quality Network. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. 2014;
- Bansal SK, Jaiswal D, Gupta N, et al. Gr/gr deletions on Y-chromosome correlate with male infertility: an original study, meta-analyses, and trial sequential analyses. *Sci Rep*. Feb 15 2016;6(1):19798. doi:10.1038/srep19798
- Li Y, Pan KJ, Wang L, Ren J. Association of gr/gr deletion in the AZFc region of Y chromosome with male infertility: a meta-analysis. *Zhonghua Nan Ke Xue*. Jun 2011;17(6):546-52.
- de Llanos M, Balleca JL, Gazquez C, Margarit E, Oliva R. High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICSI candidates. *Hum Reprod*. Jan 2005;20(1):216-20. doi:10.1093/humrep/deh582
- Shahid M, Dhillon VS, Khalil HS, Sexana A, Husain SA. Associations of Y-chromosome subdeletion gr/gr with the prevalence of Y-chromosome haplogroups in infertile patients. *Eur J Hum Genet*. Jan 2011;19(1):23-9. doi:10.1038/ejhg.2010.151
- Alimardanian L, Saliminejad K, Razi S, Ahani A. Analysis of partial azoospermia factor c deletion and DAZ copy number in azoospermia and severe oligozoospermia. *Andrologia*. Nov 2016;48(9):890-894. doi:10.1111/and.12527
- Carvalho CM, Zuccherato LW, Bastos-Rodrigues L, Santos FR, Pena SD. No association found between gr/gr deletions and infertility in Brazilian males. *MHR: Basic science of reproductive medicine*. 2006;12(4):269-273.
- Motovali-Bashi M, Rezaei Z, Dehghanian F, Rezaei H. Multiplex PCR based screening for micro/partial deletions in the AZF region of Y-chromosome in severe oligozoospermic and azoospermic infertile men in Iran. *Iran J Reprod Med*. Sep 2015;13(9):563-70.
- Rao MV, ShAh NP, Raval RJ, SOIANki PP, ShAh PS, ShAh SC. Current status of Y chromosome microdeletions: Prevalence, distribution, implication and association with male infertility in Indian men a review. *J Clin Dign Res*. 2021;15
- Petersen CG, Mauri AL, Vagnini LD, et al. The effects of male age on sperm DNA damage: an evaluation of 2,178 semen samples. *JBRA Assist Reprod*. Nov 1 2018;22(4):323-330. doi:10.5935/1518-0557.20180047