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Association of the ABCB1 gene polymorphism and infertility in azoospermia males in Iraqi Kurdish population

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Abstract

P-glycoprotein (P-gp), a product of the ABCB1 gene (ATP binding cassette subfamily B member 1) or MDR: multiple drug resistance 1 gene is expressed in various parts of the human body, like testis. The ABCB1 gene has several single nucleotide polymorphisms in its exons and introns that have been discovered. This study used DNA sequencing and polymerase chain reaction (PCR) to discover and characterize nucleotide variations in exons 27 of the ABCB1 gene in seven infertile and three normally fertile individuals. In this study, a set of primers were designed using the NCBI primer designing tool to amplify 394bp the exons 27 of ABCB1 gene and then the amplified product was sequenced by Sanger technology. Four different types of nucleotide variations have been identified in the two fertile and two infertile samples. However, one fertile sample and five infertile samples did not exhibit any changes. Three types of nucleotide variations were already described in the NCBI database they are 87509329 A>G (rs1045642 A/C/G/T) in exon 27, 87509216 A>C (rs2235047 A/C/G) and 87509195 G>A (rs2235048 G/A/C) both in intron 27. The final nucleotide change that was detected in this study was 87509240 A>T in intron 27 with heterozygous allele type (A and T allele) that was undefined in the NCBI database and consider a new recording nucleotide variation. According to the results of this study, no obvious associations have been observed between the nucleotide polymorphisms in exon 27 and infertility in the Iraqi Kurdish population in Erbil city.

Keywords

ABCB1 gene polymorphism, Male infertility, PCR technique, DNA sequencing, Nucleotide change.

Cover Page Footnote

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ORIGINAL ARTICLE

Association of the *ABCB1* Gene Polymorphism and Infertility in Azoospermia Males in Iraqi Kurdish Population

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Abstract

The P-glycoprotein (P-gp), a product of the *ABCB1* gene (ATP binding cassette subfamily B member 1) or MDR: multiple drug resistance 1 gene, is expressed in various parts of the humans body, like testis. The *ABCB1* gene has several single nucleotide polymorphisms in its exons and introns that have been discovered. This study used DNA sequencing and polymerase chain reaction (PCR) to discover and characterize nucleotide variations in exons 27 of the *ABCB1* gene in seven infertile and three normally fertile individuals. In this study, a set of primers were designed using the NCBI primer designing tool to amplify 394bp the exons 27 of *ABCB1* gene and then the amplified product was sequenced by Sanger technology. Four different types of nucleotide variations have been identified in the two fertile and two infertile samples. However, one fertile sample and five infertile samples did not exhibit any changes. Three types of nucleotide variations were already described in the NCBI database they are 87,509,329 A > G (rs1045642 A/C/G/T) in exon 27, 87,509,216 A > C (rs2235047 A/C/G) and 87,509,195 G > A (rs2235048 G/A/C) both in intron 27. The final nucleotide change that was detected in this study was 87,509,240 A > T in intron 27 with heterozygous allele type (A and T allele) that was undefined in the NCBI database and consider a new recording nucleotide variation. According to the results of this study, no obvious associations have been observed between the nucleotide polymorphisms in exon 27 and infertility in the Iraqi Kurdish male population in Erbil city.

Keywords: *ABCB1* gene polymorphism, Male infertility, PCR technique, DNA sequencing, Nucleotide change

1. Introduction

Infertility affects nearly 7% of men in the general population, with genetic disorders, including both chromosomal and single-gene alterations, accounting for at least 15% of cases. Genetic causes can be identified in all significant etiologic categories of male infertility (pre-testicular, testicular, and post-testicular forms), and genetic tests have become routine diagnostic procedures in certain patient groups [1]. In patients who have 5−10 spermatozoa/ml in their seminal fluid, genetic testing (karyotype and Y chromosome microdeletion analyses) is recommended [2]. After genetic testing, the aetiology of approximately 40% of primary testicular failure remains unknown, and the condition is referred to as 'idiopathic infertility.' The search for 'hidden' genetic factors, particularly polymorphisms, in an idiopathic infertile patients was stepped up in the late 1990s after this approach proved successful in some other complex multifactorial diseases [3,4]. Beginning in 2009, novel approaches such as single nucleotide polymorphism (SNP) array, comparative genomic hybridization-array (array-CGH), and next generation sequencing (NGS) yielded valuable data also on rare genetic variations [5].

In humans the gene *ABCB1* (ATP binding cassette subfamily B member 1) is located on chromosome 7 (7q21.12) with nucleotide location (GenBank record) NC_000007.14 (87, 503, 017−87,713,295) about 210,279 nucleotides length. The *ABCB1*gene is a protein-coding gene consisting of 32 exons and encodes to a protein with 1280 amino acids length. This gene produces a membrane-associated protein

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that belongs to the superfamily of ATP-binding cassette (ABC) transporters. Different substances are transported across extracellular and intracellular membranes by ABC proteins. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). The MDR/TAP subfamily contains this protein as a member. Members of the MDR/TAP subfamily are implicated in multidrug resistance. It is responsible for decreased drug accumulation in multidrug-resistant cells and often modulates the development of resistance to anticancer drugs (https://www.ncbi.nlm.nih.gov/gene/5243#summary).

PGP is expressed in different parts of the human body, like the brain, kidneys, liver, gastrointestinal tract, testis and placenta (Löscher W., Potschka, 2005). In the testis, PGP has been shown to interact with the tight junction protein zonula occludens 1 (ZO-1), focal adhesion kinase (FAK), junctional adhesion molecule A (JAM-A), claudin-11 and occludin, playing an essential role in the restructuring of the blood-testis barrier (BTB) during spermatogenesis [6,7].

In the human testes, ABCB1 protein (multidrug resistance protein-1) (P-gp) is mainly expressed at the luminal surface of capillary endothelial cells, thus protecting testes from possibly toxic chemicals. P-gp is also expressed in Sertoli cells, Leydig cells, and testicular macrophages, although it does not seem to be expressed in populations of germ cells [8,9].

The current investigation aimed to find out the association between the nucleotide variations in exons 27 of *ABCD1* gene with infertility in seven infertile and three fertile males in the Kurdish population of Erbil, Iraq's Kurdistan Region.

2. Materials and methods

2.1. Patients and controls

The present study was authorized by the Research Ethics Committee at the College of Science, Salahaddin University-Erbil (SUE), Iraq and informed permission was obtained from each subject (participants). This present research was carried out on 10 Iraqi Kurdish infertile males in Erbil. The attendees included seven azoospermia (based on semen fluid analysis results [10] and three normal fertile males as a control group. The ages of the participants ranged from 30 to 48.

2.2. Molecular methods

2.2.1. Genomic DNA extraction

For Genomic DNA extraction, a quantity of two ml of venous blood was obtained from each person involved using a sterilizing syringe and collected in an anticoagulant EDTA tube. Genomic DNA was extracted using a commercial kit (Genomic DNA Mini Kit, Geneaid, Taiwan), and the procedures were carried out in an accordance with the manufacturing standards. Using a NanodropTM 1000 spectrophotometer, each genomic DNA sample's quality and amount were evaluated (Thermo Scientific, USA).

2.2.2. Primer design for PCR technique

To explore the relationship between infertility and the nucleotide alterations in exon 27 of the *ABCB1* gene, the exon 27 amplified (394 bp) using newly designed primers, according to the primer-design tool on the NCBI website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Forward primer sequence (5′-3′) AGTGTGGCCAGATGCTTGTA Tm: 59.31 °C and GC: 50,00% and Forward primer sequence (5′-3′) TGCTGGTCCTGAAGTTGATCTG Tm: 60.29 °C and GC: 50,00%.

2.2.3. Sample preparation for PCR

For every sample, the PCR mixtures were prepared with a final volume 50 µl containing 25 µl of 2X of Master Mix (AMPLIQON), 2.5 µl of each primer (Forward and Reverse primer) with 10 pmol/µl concentration (Macrogen; LIGO), and 5 µl of genomic DNA as a DNA template, the mixture volume fulfilled to 50 µl with sterile nuclease-free water. The thermal cycler condition program for amplification consisted of 1 cycle of 95 as Initial denaturation, 35 cycles: 95 °C for 30 s (denaturation), 55 °C for 30sec (annealing), 72 °C for 45 s (Extension), and final extension 72 °C for10 min by the use of a thermal cycler machine (Alpha thermal Cycler; code: AC196) in molecular genetics unit of Scientific Research Center/Salahaddin University/Erbil.

2.2.4. Gele electrophoresis

Agarose gel electrophoresis was used to visualize the amplified PCR products. Five microliters of each amplified PCR product were separated over a 1.5% agarose gel for 50 min, while the gel also included 0.5 µL of ethidium bromide in 100 ml of agarose solution [11]. A 100bp DNA ladder (GeneDirex) was used to evaluate the PCR results and confirm the size of each amplicon. The DNA bands on the gel were observed using a gel documentation system with a UV light (Proxima 2500 Isogene Life Science, Netherlands).

2.2.5. Sanger sequencing

The sequencing has been conducted by the big dye terminator method at Macrogen Company (South Korea, Seoul).

2.2.6. Sequence alignment

The Basic Local Alignment Search Tool (BLAST) (Human Genomic plus transcript) belong National Center for Biotechnology Information (NCBI) had been applied (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id = GCF 000001405.40) to assess and discover the mismatch or similarity of the sample sequences with the database sequence and evaluate the statistical significance of matches.

2.2.7. Submission of DNA sequence results to the NCBI BankIt

The DNA sequence results of all samples were submitted to the NCBI BankIt for recording the results and taking the gene bank accession number for all DNA sequences.

3. Results and discussion

The amplified PCR product for all samples showed in Figure 1, all samples had the same uniform band size of 394bp. Generally, in this investigation, all 10 samples showed four different types of nucleotide changes, as shown in Tables 1 and 2.

The NCBI primer designing tool was utilized in the current study to design a novel primer to amplify exons 27 of the *ABCB1* gene (394bp) to do DNA sequencing and detect any nucleotide changes in the infertile azoospermia males and normal fertile males in the Kurdish population then comparing that result between two groups.

In this study, the sequencing samples were aligned using the NCBI-BLAST tool to look for any nucleotide mismatches. In addition, this helps to pinpoint where any potential mutations or polymorphisms might be in the samples. For a precise search for nucleotide mismatches, each DNA sequence sample was subjected to the alignment process separately.

In this investigation, four different kinds of nucleotide alterations were detected in the two fertile and two infertile samples. Although, five of the infertile samples and one of the fertile samples did not show any alterations. Among the four nucleotide changes has been detected in this study three of them were already described in the NCBI database they are 87,509,329 A > G (rs1045642 A/C/G/T) in exon 27, 87,509,216 A > C (rs2235047 A/C/G) and 87,509,195 G > A (rs2235048 G/A/C) both in intron 27. While the fourth nucleotide change was 87,509,240 A > T in intron 27 with heterozygous allele type (A and T allele) that was undefined in NCBI database Tables 1 and 2.

The variant rs1045642 in exon 27 is normally present in four types of allele (A/C/G/T allele) among individuals without specific clinical significance according to NCBI ClinVar database, in the present study this variant (G allele) nearly has been detected with similar frequency in both fertile group (33.3%) and infertile (28.6%) according to this study. Besides that, in a study by which conducted on 162 infertile male patients and 191 healthy males, they found associations between the rs1045642 polymorphism and male infertility [12].

In this study, the variant 87,509,216 A > C (rs2235047 A/C/G) was detected in just one normal sample (C allele) and not in infertile samples, but the variant 87,509,195 G > A (rs2235048 G/A/C) detected in one fertile (33.3%) and one infertile (14.3%) sample, this result suggesting no relationship between the two variants with infertility in Kurdish population. Similarly, the clinical significance of both variants has not been reported in NCBI ClinVar database.

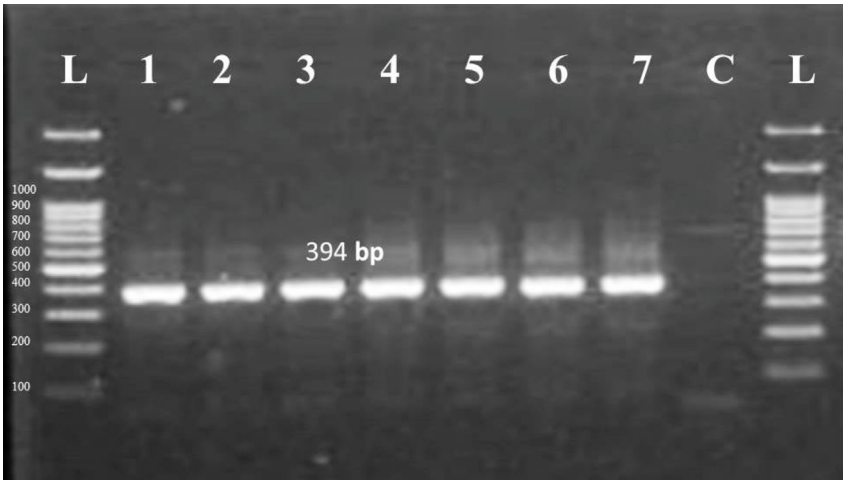

Figure 1. Agarose gel electrophoresis 1.5% (1X TBE). Lane L represent 100bp size DNA ladder marker, Lane 1–7 represents samples of PCR product 394bp of the exon 27 of ABCB1 gene. Lane C: negative control.

Table 1. The nucleotide variation and allele type among three normally fertile and seven infertile Kurdish males in ABCB1 gene's exons 27 and introns 27.

Group	Sample No.	NCBI GenBank accession number	Age /Year	Nucleotide change and location on *ABCB1* gene	Location on *ABCB1* gene	Variation type	Allele type (zygosity)
Normal Control	1	OR130483	38	87,509,329 A > G	Exon 27	rs1045642 A/C/G/T	Homozygous
				87,509,240 A > T	Intron 27	Undefined	Heterozygous
				87,509,216 A > C	Intron 27	rs2235047 A/C/G	Homozygous
				87509195G > A	Intron 27	rs2235048 G/A/C	Heterozygous
	2	OR130484	48	87,509,240 A > T	Intron 27	Undefined	Heterozygous
	3	OR130485	46	Non			
Infertile patient	1	OR130486	47	87,509,329 A > G	Exon 27	rs1045642 A/C/G/T	Homozygous
				87509195G > A	Intron 27	Undefined	Heterozygous
	2	OR130487	38	Non			
	3	OR130488	39	Non			
	4	OR130489	32	Non			
	5	OR130490	34	Non			
	6	OR130491	30	non			
	7	OR130492	32	87,509,329 A > G	Exon 27	rs1045642 A/C/G/T	Homozygous

Table 2. The frequency of nucleotide variations in the ABCB1 gene's exons 27 and introns 27 in three normally fertile and six infertile Kurdish males.

No.	Nucleotide Changes	Three Fertile Samples (%)	Seven Infertile Samples (%)
1	87,509,329 T > G rs1045642 A/C/G/T	1 (33.3%)	2 (28.6%)
2	87,509,240 A > T Undefined	2 (66.6%)	Non
3	87,509,216 A > C rs2235047 A/C/G	1 (33.3%)	Non
4	87509195G > A rs2235048 G/A/C	1 (33.3%)	1 (14.3%)

In a recent meta-analysis conducted by Sohail et al. (2023), the objective was to examine the potential association between *MDR1* gene polymorphisms and male infertility. The findings of their investigation suggest that *MDR1* gene polymorphisms may not serve as a significant risk factor for male infertility. These results meet with the outcomes of this study [13].

The most interesting detection in this study is nucleotide changes in position 87,509,240 in an intron 27 (A > T allele) that were detected only in two normal fertile samples, this change has not been reported in NCBI ClinVar database, which considering a new recording nucleotide changes in Kurdish male population.

4. Conclusion

In conclusion, appropriate pair of primers was presented suitable for sequencing the exon 27 amplified (394 bp). Generally, Four different types of nucleotide variations have been identified in the two fertile and two infertile samples, Three types of nucleotide polymorphisms had already been described in the NCBI database they are (rs1045642 A/C/G/T), (rs2235047 A/C/G) and (rs2235048 G/A/C). The fourth nucleotide change discovered in this study was 87,509,240 A > T in intron 27 that was undefined in the NCBI database. The findings of this study indicate that there is no clear correlation between male infertility in the Iraqi Kurdish population of Erbil city and the nucleotide variations in exon 27.

Conflict of interest

The author declare they have no conflicting interests.

Acknowledgement

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