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Association of Polymorphisms in *DAZL* Gene with Male Infertility

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Authors' contributions

This work was carried out in collaboration between all authors. Author OFK designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author AMA performed the experimental work. Author AAA recruited and interviewed the subjects. Author FZ managed and analyzed clinical data. Author MFS supervised experimental part and provided the space and equipments for molecular analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Objectives: To investigate the association between A260G (Thr12Ala) and A386G (Thr54Ala) single nucleotide polymorphisms (SNPs) in deleted in azoospermia-like gene (*DAZL*) and infertility in Jordanian males.
Methods: Infertile 170 patients with azoospermia or oligozoospermia and 176 fertile subjects were recruited in the study. *DAZL* SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP).
Results: The data showed that the A260G SNP is common in the Jordanian population with frequency of 10.3% for 260G mutant allele. However, the A386G SNP is absent in the studied population. No significant association was found between the examined SNPs in *DAZL* gene and men infertility.

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Conclusion: The A260G and A386G polymorphisms of DAZL seem to play no role in men infertility in Jordanian population.

Keywords: Deleted in azoospermia-like gene; single nucleotide polymorphism; Jordan; infertility; male.

1. INTRODUCTION

Infertility is a worldwide health problem that affects about 10–15% of couples, with approximately equal percentage from both sexes [1]. Several investigations have reported that at least 10% of males with azoospermia or oligozoospermia have mutations in the deleted in azoospermia (*DAZ*) genes family that consists of 3 members: *DAZ* gene on the Y chromosome, *DAZ*-like (*DAZL*) gene on the 3p24, and *BOULE* gene on the 2q33 [2,3]. All *DAZ* genes code for RNA-binding proteins that are expressed in germ cells [4]. During meiosis, DAZL protein transferred from the nucleolus of spermatogonia cells into the cytoplasm, which suggests a dynamic role of DAZL protein during gametes formation [5]

Similarity between *DAZL* gene and *DAZ* genes on the Y chromosome in the coding region reaches up to 83% [4]. *DAZL* knockout mice are infertile due to absence of gametes in both male and female mice [5]. In addition, transfer of human *DAZ* gene to *DAZL* knockout mice partially rescues the loss of germ cells, which indicates a high conservation in function between *DAZ* and *DAZL* gene [6]. Similarly, interference with *DAZL* homologues causes meiotic arrest during oogenesis in *Caenorhabditis elegans* and prevents primordial germ cell differentiation in *Xenopus* [7,8]. In human, infertile men with testicular failure have low level of mRNA of the *DAZL* gene [9] and some individuals with *DAZL* polymorphisms were susceptible to the spermatogenic failure [10]. These findings suggest that *DAZL* is a specific autosomal gene for germ cell and may be associated with human male infertility.

Recently, several functional single-nucleotide polymorphisms (SNPs) in the *DAZL* gene have been reported. For example, the *DAZL* A260G SNP in exon 2 that replaces threonine by alanine at position 12 (T12A) is associated with sperm count and motility in infertile men [11]. *DAZL* A386G in exon 3 that also replaces threonine by alanine at position 54 (T54A) in the RNA recognition motif of DAZL protein is associated with spermatogenic failure in Taiwanese population [10,12]. The association between these polymorphisms and male infertility is still controversial [13-17] and more studies are required. Therefore, in this study, we investigated the distribution of the *DAZL* A260G and A386G SNPs in Jordanian males. In addition, we evaluated the association of these SNPs with male infertility.

2. METHODOLOGY

2.1. Subjects and Blood Sampling

One hundred and seventy unrelated infertile men with oligozoospermia (sperm count; 5–20 million/mL, n=47), severe oligozoospermia (sperm count <5 million/mL, n=87) and azoospermia (n=36) were recruited from King Abdullah University Hospital and Al-Hussein Medical Center in North and Middle of Jordan respectively [13-15]. Patients with recognizable causes of male infertility such as chromosomal abnormalities, hypogonadotrophic hypogonadism, obstructive azoospermia and infections were excluded from study [16]. In addition, 176 fertile control men (semen analysis >20 million sperms/ml)

were matched to infertile men for geographical origin [18,19]. Clinical and demographic characteristics of subjects were collected using a questionnaire that covers fertility parameters, medical history, chronic diseases and social habits. A written consent form was obtained from all participants as required by Jordan University of Science and Technology - Institutional Review Board. Blood specimens (3ml) were collected in EDTA tubes from all subjects for genomic DNA extraction.

2.2. Genotyping of DAZL SNPs

Genomic DNA was extracted using commercially available kit (Gentra Puregene Blood Kit, Germany) according to the manufacturer instruction. The *DAZL* A260G and A386G genotypes were determined by PCR amplification and subsequent digestion with DdeI and AluI restriction enzymes respectively [10]. The set of primers for amplification of A260G was: forward (5'CCT GTG TAT CTA ATT ATG ATG3') and reverse (5'CCT TAA GTT TGT AAC AGG GCC3'), and for amplification of A386G was: forward (5'GAA TGC TGA ATT TTT ACT CTT GAA G3') and reverse (5'CTC TAT ACG TGG CTA GAG TTC3'). The PCR condition and cycling were: initial denaturation at 95ºC for 4 min followed by 30 cycles of 94ºC for 60s, 55ºC for 60s and extension at 72ºC for 60s and final extension at 72ºC for 7 min. PCR products were detected on 2% agarose electrophoresis, confirming the presence of a 264 bp fragment for A260G and a 181 bp fragment for A386G. In the restriction reaction, 10ul of PCR products were digested at 37ºC for 16 hours with 5 units of *DdeI* and *AluI* enzymes for A260G and A386G respectively. The digested fragments were separated with 2.5% agarose gel and visualized by staining with ethidium bromide. Materials from the 260A allele are not cut by *DdeI* and remain as a 264 bp product whereas the 260G allele is cut by the enzyme to give 67 bp and 197 bp fragments. However, materials from the 386A allele are cut by *AluI* to give 115 and 66 bp products whereas the 386G allele is cut by the enzyme to give 115, 53 and 13 bp fragments.

2.3 Statistical Analysis

Statistical analysis was computed by using the statistical package for social studies SPSS. Chi-square test was used to evaluate the genotype distribution and allele frequencies of the studied polymorphisms. A *P-value* of < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSIONS

A total of 346 Jordanian men were recruited from North and Middle of Jordan to participate in the study. The subjects were 170 infertile men and 176 fertile controls. The main participant characteristics are summarized in Table 1. The infertile men were different from fertile men in sperm count, sperm motility and sperm morphology. However, no differences were found between the two groups in terms of semen viscosity, smoking habit and body mass index. About 21.2% of the infertile men were azoospermic, 51.2% were severe oligospermic and 27.6% were oligospermic.

Table 1. Demographic and clinical characteristics of participants

Variable	Fertile men	Infertile men	*P* value
Age (years: mean ± SD)	36.46 ± 6.6	38.2 ± 9.1	0.13
Body mass index (kg/m2) (%)			
≤ 24.9	26.9	26.1	
25 – 34.9	67.1	60.9	
≥ 35	6.0	13.0	0.41
Smoking (%)			
Yes	65.9	69.1	
No	34.1	31.9	0.49
Semen analysis (%)			
Sperm count ≤ 20 (million/mL)	0	100	<.001
Motility (grade a + b):			
≥ 50	65.9	7.5	
< 50	34.1	92.5	<.001
Viscosity :			
≤ 30 min	61.5	72.6	
> 30 min	38.5	27.4	0.09
Normal morphology			
≥ 30%	37.5	15.0	
< 30%	62.5	85.0	<.001

Table 2 shows the summary of genotype and allele frequencies of the *DAZL* A260G SNP. The G allele of A260G SNP (Fig. 1) was found in both the fertile and infertile men at frequency of 12.5% and 10.3% respectively, while the frequencies of the A allele was 87.5% in fertile men and 89.7% in infertile men. No significant statistical difference was demonstrated in the distribution of the G and A alleles among the fertile and the infertile males (*P*=.87). The genotype frequencies of A260G were also not different between the two groups (*P*=.62, Table 2). According to Hardy Weinberg analysis, all genotypic groups of A260G SNP were in equilibrium. In addition, the frequency of the G allele of A260G SNP was within the range detected in other populations (3.14-18.75%, Table 3).

The A386G SNP was absent in the Jordanian population. The frequency of the A allele was 100% in both fertile and infertile subjects. The A386G SNP was absent in most of the examined world populations (Table 3).

Spermatogenesis is a complex process controlled by several genes located in both autosomal and sex chromosomes [20]. About 10% of infertile men have complete or partial deletions of the *DAZ* gene cluster [2,3]. The role of *DAZL* in spermatogenesis is supported by its testis specific expression and its high homology to *DAZ* gene [21,22]. Recently, many pieces of evidence suggested that *DAZL* gene may play a role in germ cell development. For example, in *Caenorhabditis elegans*, inactivation of *DAZL* was associated with meiotic arrest in oogenesis [7]. In mice, knockout of the *DAZL* homolog leads to loss of germ cells in both sexes [5]. Finally, in human beings, low mRNA transcript levels of the *DAZL* gene was found in infertile men with testicular failure [9].

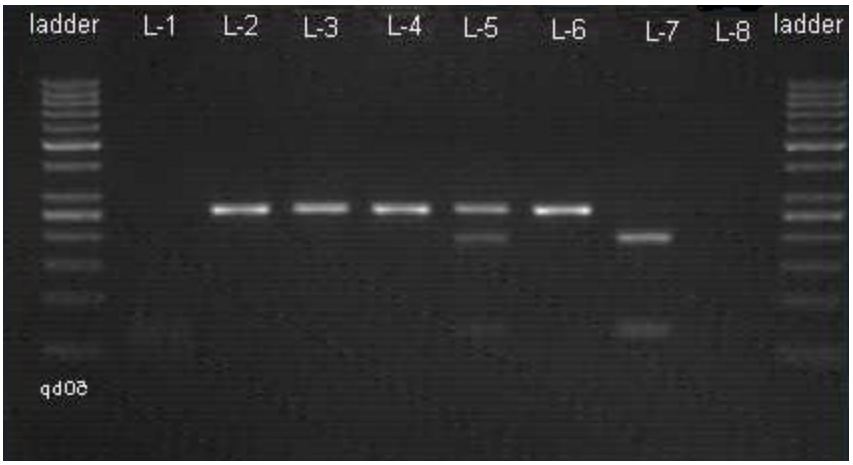

Fig. 1. Gel showing different genotypes of DAZL A260G polymorphism.

The amplified PCR fragment that contains A260G polymorphism (264 bp) was digested using DdeI restriction enzyme, separated using electrophoresis and visualized using ethidium bromide. L-1 and L-8 are negative control. L-2, L-4 and L-6 represent PCR product of three samples and L-3, L-5 and L-7 are the digest product of each sample, respectively. L-3 is the wild homozygous uncut AA genotype (264bp). L-5 represents the heterozygous AG genotype (264bp, 197bp and 67bp). L7 represent the mutant homozygous GG genotype (197bp and 67bp). 50bp DNA ladder in both end was used.

Table 2. Genotypes and alleles frequency of A260G in fertile and infertile men

Genotypes and Alleles	Fertile men(176) N (%)	Infertile men(170) N (%)	*P*-value
AA	137(77.9)	138(81.2)	
AG	34(19.3)	29(17.1)	
GG	5(2.8)	3(1.7)	0.67
Allele A	308(87.5)	305(89.7)	
Allele G	44(12.5)	35(10.3)	0.82

Table 3. Summary of the distribution of DAZL SNPs among various populations

Variable	A260G		A386G		References
	Allele A	Allele G	Allele A	Allele G	
Jordanian	87.5	12.5	100	0	Current study
Indian	91.5	8.5	100	0	[14,29]
Taiwanese	96.85	3.14	99.48	0.52	[15]
Chinese	81.25	18.75	-	-	[23]
Japanese	93.08	6.92	100	0	[17]
Italian	87.34	12.66	100	0	[13]
German	87.88	12.12	100	0	[16]

The results showed that the A260G SNP is common in the Jordanian population. The frequency of the mutant G allele of A260G SNP is 10.3%. Similar frequencies were also detected in populations like Italian and German [13,16]. However, lower abundance of the G allele was reported in populations like Indian, Taiwanese and Japanese [11,14,17]; whereas it is slightly higher in Chinese [23]. The A386G SNP is absent in Jordanian population. This

SNP was reported in low frequency in Taiwanese [10] while it was absent in other populations [13-17]. Despite these ethnic variations in the distribution of *DAZL* SNPs, in the majority of the populations A260G is common while A386G SNP is rare or absent.

The results showed that the examined SNPs in *DAZL* gene were not associated with male infertility. This is in agreement with most studies that examined the association between *DAZL* polymorphisms and male infertility. For example, lack of association between A260G SNP and male infertility was reported in Indian, Chinese, Japanese, Italian and German populations [13-17]. On the other hand, the A260G SNP was associated with an increase in sperm count, whereas the A386G SNP has been suggested to be associated with spermatogenic failure in Taiwanese population [10,11,24]. The A386G SNP was not detected in many populations including Jordanian (see discussion above).

We limited this study to *DAZL* gene and its relationship to infertility in Jordanian population. However, the genetic causes of infertility are heterogeneous and include chromosomal aneuoploidy, chromosomal rearrangements, gene deletions and point mutations in several genes [20,25]. Covering all such factors requires extensive efforts and years of investigations. In addition, non genetic factors such as metabolic syndrome, obesity, and others might be related to infertility [26-28]. A national project that investigates such causes is demanding since a considerable fraction of the Jordanians suffers from this problem.

4. CONCLUSION

This study revealed that the polymorphisms of *DAZL* gene (A260G and A386G) are not risk factors for male infertility in the Jordanian population.

ETHICAL APPROVAL

The study procedures were approved by Jordan University of Science and Technology - Institutional Review Board and ethical committees at the participating hospitals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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