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DAZL A386G gene mutation and male infertility: A genetic association analysis of Asian population

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ABSTRACT

Genetic susceptibility has a prominent role in infertility. This study was proposed the association of Deleted in Azoospermia-Like (DAZL) A386G gene transition with male infertility in an Iranian population which followed by a meta-analysis in Asian population. In the case-control study we collected blood samples from 100 idiopathic infertile and 100 healthy fertile men. After DNA extraction, DAZL A386G genotyping was performed by PCR-RFLP method. In meta-analysis, we found eligible papers by searching in standard databases. Case-control study indicated that there is no significant association between DAZL A386G and male infertility in study population. However in meta-analysis, we found a significant association between DAZL A386G and male infertility in G *vs*. A (OR= 8.33, 95% CI= 3.56-19.46, $P < 0.001$), AG *vs*. AA (OR= 7.60, 95% CI= 3.24-17.82, $P < 0.001$), and AG+GG vs. AA (OR= 8.13, 95% CI= 3.47-19.07, $P < 0.001$) genetic models within Asian population. Therefore, we concluded that DAZL A386G can be considered as a possible risk factor for susceptibility to male infertility within Asian population. However, further studies with larger sample sizes are required to obtain more accurate data.

KEY WORDS: MALE INFERTILITY; DAZL; GENETIC POLYMORPHISM; META-ANALYSIS

INTRODUCTION

Infertility refers to the inability to conceive after at least 12 months of regular unprotected intercourse. It is an important health issue that affects 10-15% of couples, worldwide. Almost half of infertility causes are related to male factors (Agarwal et al., 2015). Environmental factors, life style, and genetic background are involved in male infertility. However, about half of male infertility cases remain unknown which is called idiopathic infertility (Neto et al., 2016). Numerous elements could involve in idiopathic infertility such as DNA damage of sperm and other genetic abnormalities. Recently, several genetic association studies are developed for evalua-

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tion of genetic polymorphisms effects on male infertility risk In these genetic association studies, an abundant attention has been focused on the Deleted in Azoospermia-Like (DAZL) gene (Treulen et al 2015 and Karimian and Colagar, 2016a).

This gene is autosomal homologue of DAZ, which is deleted in approximately 10% of azoospermic or oligozoospermic men (Nagafuchi et al., 1993; Reijo et al., 1995), and nominated as a male infertility risk. This gene is expressing during spermatogenesis in the germ cells and localizing in spermatogonia and gonocytes (Reynolds et al., 2005). Some evidences showed that DAZL gene has an essential role in spermatogenesis process and it is regulating mRNA expression as a translational activator (Ruggiu et al., 2000).

There are two common single nucleotide polymorphisms (SNPs) in DAZL gene including SNP260 and SNP386. The SNP260 (A260G) is located on exon 2 and results in threonine to alanine substitution at codon 12 (Thr12Ala). Whereas, SNP386 (A386G) is located on exon 3 and lead to substitution of threonine with alanine at codon 54 (Thr54Ala) (Zhang et al., 2014). In this study we investigated the association of SNP386 and male infertility in an Iranian population and followed it by a meta-analysis in Asian population.

MATERIALS AND METHODS

CASE-CONTROL STUDY

In the case-control study, we collected 100 idiopathic infertile and 100 healthy fertile, age-matched men as a case and a control groups, respectively. All subjects in case group were classified in azoospermia. The healthy controls were men with normal sperm parameters and without infertility, who had at least one child. The subjects were selected from IVF center (Kashan, Iran). The inclusion and exclusion criteria were described in our previous study, in detail (Nikzad et al., 2015).

After obtaining signed informed consent form, we got 2 ml blood sample from all subjects and collected it on the EDTA. . The genomic DNA was isolated from blood samples by DNGplus DNA extraction buffer (CinnaGen, Iran). The PCR-RFLP method was used to SNP386 genotyping as detailed previously (Teng et al., 2002). After digestion of PCR fragments by *Alu*I restriction enzyme (Fermentas, Germany), the enzymatic mixtures were electrophoresed on 12% polyacrylamide gel and stained by AgNO3. PCR products in wild samples were digested to 115- and 66-bp fragments while in mutant samples the 66-bp band was digested to 53- and 13-bp fragments. Therefore, heterozygote samples were contained four following fragments: 115-, 66-, 53-, and 13-bp.

META-ANALYSIS

We searched for all studies that investigated the correlation of DAZL SNP386 mutation with male infertility in Asian population. To find these studies, we performed an accurate search via Google Scholar, PubMed, and Scince-Direct databases until October 2016. For search process, we used the following words: “male infertility”, “DAZL”, “polymorphism”, “SNP386”, “A386G”, and “Thr54Ala”. Also, some studies were found by assessment of reference list of articles which collected in electronic search. The studies which met the following criteria were included in meta-analysis: 1. relation of DAZL A386G and male infertility risk. 2. case-control studies. 3. enough data for calculation of odds ratio (OR) and its 95% confidence interval (CI). Some information including the name of authors, publication year, and genotype and allele frequencies were extracted from included studies.

STATISTICAL ANALYSIS

In the case-control study, OR with 95% CI was calculated for each alleles and genotype. To compare the differences in allele and genotype frequencies between case and control groups, we employed a Chi-square test. The *P*-value less than 0.05 ($P< 0.05$) has been considered as statistically significant. These statistical analyzes were performed by SPSS software version 19.

Meta-analysis was done in five following genetic models: 1- G vs. A (allelic), 2- GG vs. AA (co-dominant), 3- AG vs. AA (co-dominant), 4- AG+GG vs. AA (dominant), and 5- GG vs. AA+AG (recessive). A Chi square based ‘Q’ test and I^2 score was employed to calculate the heterogeneity (a *P*-value less than 0.1 was considered as statistically significant) (Higgins et al., 2003). In the presence of true heterogeneity, the random-effect model was applied to pool data, but in the absence of significant heterogeneity, the fixed-effect model was used (Der Simonian et al., 1986; Huedo et al., 2006). For sensitivity analysis, each study was eliminated from the meta-analysis at a time to evaluate the magnitude of impact on the total estimate. Egger’s test and Begg’s funnel plot were employed to evaluate the possible publication bias (Begg and Mazumdar 1994; Egger et al., 1997). Two following software including: Open Meta-analyst and Comprehensive Meta-analysis were used for statistical meta-analysis.

RESULTS

DISTRIBUTION OF A386G IN OUR POPULATION STUDY

The allele and genotype frequencies of DAZL A386G are given in table 1. Our data revealed that A386G mutation

Table 1: Genotype and allele frequencies of A386G in cases and controls.

Genotype/Allele	Control (%) (n= 100)	Cases (%) (n= 100)	OR (95% CI)	P-value
AA	100 (100%)	97 (97%)	-	-
AG	0 (0%)	2 (2%)	5.15 (0.24-108.76)	0.292
GG	0 (0%)	1 (1%)	3.09 (0.12-76.84)	0.491
AG+GG	0 (0%)	3 (3%)	7.22 (0.37-141.53)	0.193
A	200 (100%)	196 (98%)	-	-
G	0 (0%)	4 (2%)	9.18 (0.49-171.71)	0.138

OR, Odds Ratio; CI, Confidence Interval.

Table 2: Characteristics of included studies

Country	Genotype frequencies						Allele frequencies				Reference
	Control			Case			Control		Case		
	AA	AG	GG	AA	AG	GG	A	G	A	G	
China	114	2	0	121	21	0	230	2	263	21	Teng et al., 2002
Japan	131	0	0	234	0	0	262	0	468	0	Yang et al., 2005
India	349	1	0	656	4	0	699	1	1316	4	Thangaraj et al., 2006
China	189	2	0	205	25	1	380	2	435	27	Teng et al., 2006
China	53	0	0	144	0	0	106	0	288	0	Wen et al., 2007
India	140	0	0	147	0	0	280	0	294	0	Poongothai et al., 2008
China	40	0	0	192	0	4	80	0	384	8	Wang, 2009
India	199	1	0	165	0	0	399	1	330	0	Singh and Raman, 2009
China	175	0	0	173	0	0	350	0	346	0	Ye et al., 2013
Jordan	176	0	0	170	0	0	352	0	340	0	Khabour et al., 2013
Iran	100	0	0	97	2	1	200	0	196	4	This study

is absent in control group. Although, we found 2 heterozygote and 1 mutant homozygote in case group, but, statistical analysis revealed that there are no significant associations between AG (OR: 5.15, 95%CI: 0.24-108.76, *P*= 0.292) and GG (OR: 3.09, 95%CI: 0.12-76.84, *P*= 0.491) genotypes and male infertility. In addition, carriers of G allele (AG+GG) were not at a high risk for male infertility (OR: 7.22, 95%CI: 0.37-141.53, *P*= 0.193). Also, allelic analysis revealed that G allele is not a risk factor for male infertility (OR: 9.18, 95%CI: 0.49-171.71, *P*= 0.138).

META-ANALYSIS

After search and screening of studies for meta-analysis, we found 10 eligible papers. From these 10 arti-

Table 3: Association results in the meta-analysis

A) Association results

G *vs.* A		GG *vs.* AA		AG *vs.* AA		AG+GG *vs.* AA		GG *vs.* AA+AG	
OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
8.33 (3.56-19.46)	< 0.001	1.26 (0.45-3.55)	0.659	7.60 (3.24-17.82)	< 0.001	8.13 (3.47-19.07)	< 0.001	1.23 (0.44-3.46)	0.695

B) Heterogeneity and publication bias results

G *vs.* A			GG *vs.* AA			AG *vs.* AA			AG+GG *vs.* AA			GG *vs.* AA+AG		
Ph	I^2	*Pe*	*Ph*	I^2	*Pe*	*Ph*	I^2	*Pe*	*Ph*	I^2	*Pe*	*Ph*	I^2	*Pe*
0.357	0%	0.096	0.999	0%	0.112	0.285	0%	0.078	0.296	0%	0.048	0.999	0%	0.246

OR: Odds ratio, CI: Confidence interval
Ph: Pheterogeneity (p< 0.1) was considered as a significant difference. Pe: PEgger (p< 0.05) was considered as a significant difference

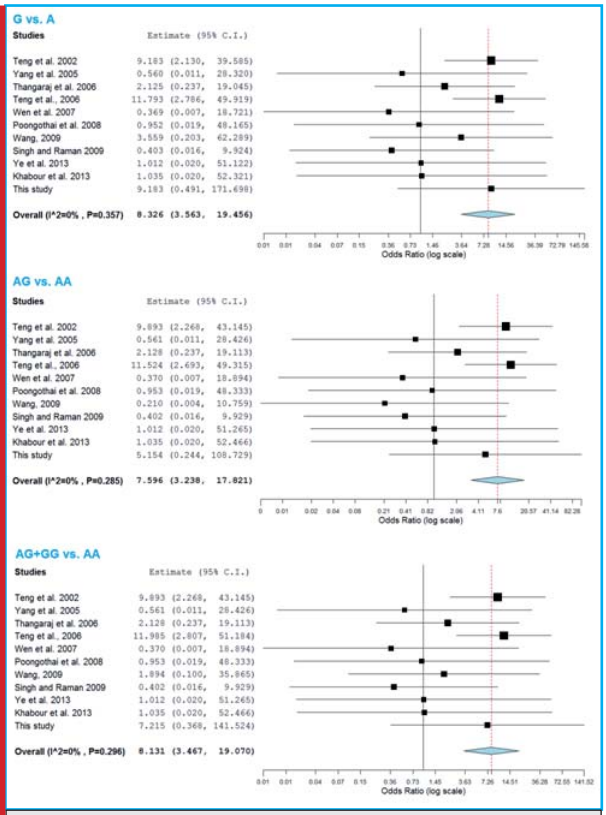

FIGURE 1. Forest plot for the association of DAZL A386G transition with male infertility.

cles, 5 studies are related to Chinese population (Table 2). Results of meta-analysis are presented in table 3. Our data indicated that there is significant association between DAZL A386G and male infertility in G *vs.* A (OR= 8.33, 95%CI= 3.56-19.46, $P< 0.001$), AG *vs.* AA (OR= 7.60, 95%CI= 3.24-17.82, $P< 0.001$), and AG+GG *vs.* AA (OR= 8.13, 95%CI= 3.47-19.07, $P < 0.001$) genetic models (Figure 1). But, association between GG *vs.* AA and GG *vs.* AA+AG genetic models and male infertility was not significant. Also, in the meta-analysis we did not find true heterogeneity in (G *vs.* A: $P_{heterogeneity}= 0.357$, $I^2= 0\%$; GG *vs.* AA: $P_{heterogeneity}= 0.999$, $I^2= 0\%$; AG *vs.* AA: $P_{heterogeneity}= 0.285$, $I^2= 0\%$; AG+GG *vs.* AA: $P_{heterogeneity}= 0.296$, $I^2= 0\%$; GG *vs.* AA+AG: $P_{heterogeneity}= 0.999$; $I^2= 0\%$) genetic models. Evaluation of meta-analysis regard to possible publication bias revealed that there is no publication bias in G *vs.* A ($P_{Egger}= 0.096$), GG *vs.* AA ($P_{Egger}= 0.112$), AG *vs.* AA ($P_{Egger}= 0.078$), and GG *vs.* AA+AG ($P_{Egger}= 0.246$) genetic models. But, we observed a marginal publication bias in AG+GG *vs.* AA ($P_{Egger}= 0.048$) genetic model (Figure 2). Sensitivity analysis revealed that exclusion of each study has no significant effect on overall meta-analysis (data not shown).

DISCUSSION

In this study we investigated the association of DAZL A386G gene mutation with male infertility in a case-control study and a meta-analysis approach in Asian population. We found that there was no association between DAZL A386G polymorphism and male infertility in study population. Nevertheless there were significant associations between DAZL A386G and male infertility in G vs. A, AG *vs.* AA, and GG *vs.* AA+AG genetic models within Asian population. The partial differences between results of individual studies may arise from ethnic and geographical differences. Also, it may be due to small population studied. In addition we did not observe significant publication bias in meta-analysis except in AG+GG *vs.* AA genetic model. A publication bias can due to small sample sizes or low quality. In addition, the bias may arise from greater luck of positive outcomes for publication than negatives (Stanley, 2005).

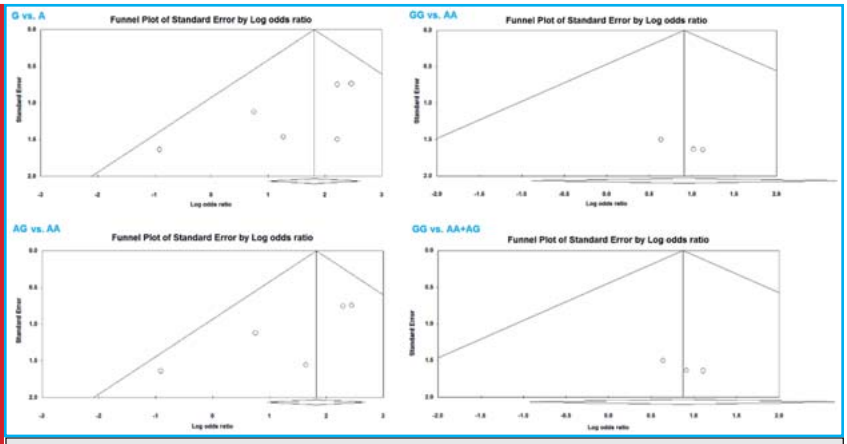

FIGURE 2. Funnel plot for association of DAZL A386G transition with male infertility.

Some possible mechanism can explain the role of Deleted in Azoospermia-Like gene in male reproductive system. Dazl gene is crucial for germ cells differentiation (Eberhart et al., 1996). For example knockout of Dazl in mice models results in the lack of production of gametes and loss of germ cells (Ruggiu et al., 1997). Another matter which could explain the essential role of DAZL gene in male fertility is the expression profile of this gene in the testis. Lin et al (2001) reported that DAZL transcripts in men with spermatogenic failure were lower than normal. They obtained testis tissues from obstructive azoosperma men with normal spermatogenesis and non-obstructive azoospermia men with abnormal spermatogenesis. Their analysis from DAZL expression revealed that there is a higher expression level of *DAZL* in obstructive azoospermic men rather than it in non-obstructive azoospermic men (Lin et al., 2001). According to this finding, evaluation of polymorphisms and mutations in the promoter of DAZL gene in azoospermic men can be considered in further studies, because functional polymorphisms in promoter region may affect gene expression (Jamali et al., 2016).

The coding SNPs, which located on exon region, could change the function and structure of protein (Raygan et al., 2016). It is predicted that about 25-30% of coding SNPs may reduce protein function (Ng and Henikoff, 2002). Also, coding SNPs can alter some other molecular aspects such as structure and function of mRNA, splicing pattern, and post-translational modifications (Karimian et al., 2015; Karimian and Colagar, 2016b). On the other hand, in silico analysis is a helpful approach to analyze the damaging effects of SNPs on the several molecular aspects (Karimian et al., 2015). Therefore this approach will be helpful for evaluation of the effects of A386G as an exonic SNP on the mRNA structure, RNA splicing, protein function, and post translational modification of DAZL. There are two meta-analysis studies about the association of A386G DAZL gene polymorphism and male infertility (Zhang et al., 2014; Chen et al., 2016).

These studies showed that there was significant association between A386G and male infertility within Asian population. But, there are some mistakes in these studies that should be considered. Zhang et al. (2014) presented the some genotypes frequencies of both of Thangaraj et al. (2006) and Poongothai et al. (2008) studies as wrong. In addition, the some genotypes frequencies of Wen et al. (2007) and Poongothai et al. (2008) were reported by Chen et al. (2016), incorrectly.

Finally there are some limitation in this study that should be mentioned. In case-control study, we did not considered the effects of gene-gene and gene-environment interactions, because these interactions may modulate the effects of A386G DAZL on male infertility. In addition, in meta-analysis we did not access to original data such as BMI and biochemical characteristics to justify the role of A386G DAZL in male infertility. Moreover, few studies are included in meta-analysis, therefore more studies with larger sample size and by considering environmental factors are necessary to obtain more accurate data.

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