

## Original Article

## Frequency of CYP1A1 Gene Polymorphisms in Infertile Men with Non-obstructive Azoospermia

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**Key words**

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**Background and Aims:** The cytochrome P450 1A1 (CYP1A1) plays a curial role in phase I metabolism of polycyclic aromatic hydrocarbons to their ultimate biologically active intermediates that have potential reproductive toxicity in men. Reproductive functions in men may be impaired by many environmental, physiologic, and genetic factors. The majority of the environmental factors are xenobiotics. Metabolic active xenobiotics exert adverse effects via covalent interactions between intermediate metabolites and cellular macromolecules such as DNA or protein.

**Materials and Methods:** Genotyping two polymorphisms, CYP1A1\*2A and CYP1A1\*2C, was done using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay in a case–control study including 105 infertile men and 104 healthy fertile subjects.

**Results:** The results showed that frequency of CYP1A1\*2A was significantly different between the patients and the controls ( $p = 0.036$ ). Analysis indicated that CYP1A1\*2A CC genotype was significantly associated with an increased risk of male infertility (OR = 5.4, CI=1.12-26.04; 95%) compared with the AA genotype. No significant association was detected between CYP1A1\*2C polymorphism and male infertility.

**Conclusions:** The CYP1A1\*2A single nucleotide polymorphism can be considered as an effective agent in azoospermia.

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## Introduction

Infertility refers to sexual intercourse without pregnancy within 12 months, and this problem affects 10-15% of couples in the United States [1]. Male factor infertility is partially or fully responsible for approximately 30-55% cases of infertility [2, 3]. Azoospermia, which is the complete absence of sperm in the ejaculate, accounts for 10-15% of male infertility cases and generally affects 1% of the male population [3-5]. One of the most important mechanisms involved in genetic studies is attention to the cytochrome P4501A1 (CYP1A1), which plays a crucial role in phase I metabolism of polycyclic aromatic hydrocarbons to ultimate biologically active intermediates. It has potential reproductive toxicity in men [6, 7]. CYP1A1, is involved in the metabolism of substrates through catalysing the hydroxylation of 17 $\beta$ -estradiol at the C-2 position [8]. The polymorphic gene CYP1A1 (CYP1A1\*2A CC genotype) encodes CYP1A1 enzyme. The catalyzes of polycyclic aromatic hydrocarbons (PAHs) are able to form DNA adducts. The DNA adducts in sperm cells can cause severe DNA damage and interfere with meiotic division during spermatogenesis. It can be related with infertility in men [9]. It has been recently reported that the genetic polymorphisms CYP1A1\*2C of xenobiotic-metabolizing enzymes may possibly play an important role in male factor infertility [10]. Cytochrome P4501A1 gene, located on chromosome 15q22-q24, is 5987-bp long and encodes 512 amino acid protein. Two polymorphisms have been identified in

CYP1A1 gene in the Han-Chinese population [11, 12] CYP1A1\*2A (T to C substitution at nucleotide 3801 in the 3'-non-coding region, rs4646903) and CYP1A1\*2C (Ile462Val, A to G substitution at nucleotide 2455 leading to an amino acid change of isoleucine to the homeostasis of male reproductive valine at codon 462 in exon 7, rs1048943). CYP1A1\*2A is most prevalent in the Asian population. T/C polymorphism has been proposed to be a functional-related site [13, 14]. These reports implied an essential role for the two polymorphisms. CYP1A1 is a key enzyme in phase I bioactivation of the series of PAHs, which have reproductive toxicity. It can be associated with the risk of male infertility. Thus, the present study analysed genotyping of CYP1A1\*2A and CYP1A1\*2C polymorphisms in infertile male referring to Yazd Reproductive Sciences Institute, to evaluate any correlation between these genes and impair spermatogenesis.

## Materials and Methods

### Subjects

One hundred and five infertile men with non obstructive azoospermia who had referred to Yazd Reproductive Sciences Institute were studied. One hundred and four fertile men were included in this study as controls. All patients and controls were Iranian. Infertile men with known causes (cytogenetic, hormonal and Y-chromosomal deletions) were excluded from the study. All cases and controls signed inform consent form and then entered to this study. The

procedure was approved by Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Blood samples were taken from all of them and DNA was extracted for genetic tests. Isolation of genomic DNA from peripheral lymphocytes was carried out using Salting out method, and the DNA stored at 4°C in the refrigerator until laboratory analysis was performed.

#### **Deoxyribonucleic acid isolation and genotyping**

Each subject donated 5 mL of blood for genomic DNA extraction. The genomic DNA was extracted from the peripheral blood lymphocytes, using salting out method [15]. The CYP1A1\*2A and CYP1A1\*2C polymorphisms were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

The primers used for CYP1A1\*2A polymorphism PCR were prepared using a 380 bp DNA synthesizer (Pishgam company, Tehran City, Iran) sequences of primers derived from the published sequence of CYP1A1 were as follows with primers:

5'-TAGGAGTCTTGTCTCATGCCT-3' and 5'-CAGTGAAGAGGTGTAGCCGCT-3' [16].

#### **CYP1A1\*2A polymorphism PCR**

The genotypes of CYP1A1 were detected using designed RFLP [16]. Briefly, the PCR reaction mixture contained approximately 1 µl of genomic DNA, 10 µl (Taq 2x Master Mix RED 1.5 mM MgCl<sub>2</sub>), 0.7 µl of a pair of primers and 16.2 µl H<sub>2</sub>O reaction using a thermal cycler (Eppendorf-Germany) was at 95°C for 10 min. to effect initial denaturation, followed by 35 cycles of denaturation at 95°C for 1 min.,

annealing at 62°C for 1 min., and extension at 72°C for 10 min.

#### **Detection of CYP1A1\*2A Polymorphism using designed RFLP**

Ten µl of PCR product was digested with *Msp I* (New England Biolabs, USA) at 37°C for 4.5 h. Restriction digestion occurred in the presence of the C allele, thus yielding fragments of 200 and 140 bp as visualized after fractionation by agarose gel electrophoresis. In the presence of the T allele, the PCR product remained intact. After digestion, the products were subjected to agarose gel (1.5%) electrophoresis, followed by safe staining. The gels were photographed using an ultraviolet light transilluminator (Figure 1).

#### **CYP1A1\*2C polymorphism PCR**

The primers used for CYP1A1\*2C polymorphism PCR were prepared using a 248 bp DNA synthesizer (Pishgam company, Tehran City, Iran). Sequences of primers derived from the published sequence of CYP1A1 were as follows with primers:

5'-TTCATGGTTAGCCCATAGATG-3' and 5'-TACAGGAAGCTATGGGTCAAC-3' [17].

The genotypes of CYP1A1 were detected using designed RFLP [17]. Briefly, the PCR reaction mixture contained approximately 5 µl of genomic DNA. The DNA concentration in the PCR reaction was between 5-50 ng/µl, 10 µl (Taq 2x Master Mix RED 1.5 mM MgCl<sub>2</sub>), 0.7 µl of a pair of primers, and 16.2 µl H<sub>2</sub>O reaction using a thermal cycler (Eppendorf-Germany) was at 95°C for 10 min. to effect initial denaturation, followed by 35 cycles of denaturation at 95°C for 1 min., annealing at 64°C for 1 min., and extension at 72°C for 10 min.

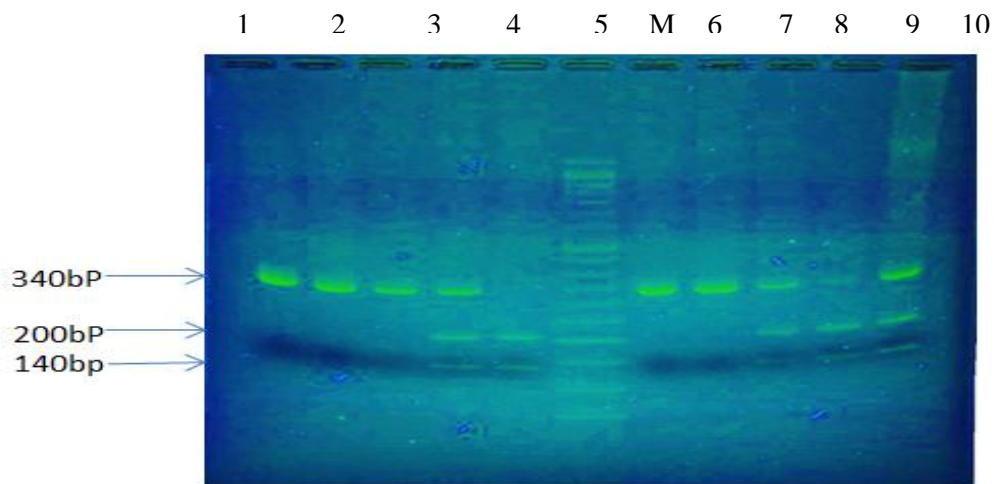
**Detection of CYP1A1\*2C Polymorphism using designed RFLP**

Ten µl of PCR product was digested with *BsrDI* (New England Biolabs, USA) at 57°C for 4.5 h. In the presence of the A allele, the PCR product was cleaved into two fragments (133 and 115 bp) while with the G allele, the original fragment remained intact. After digestion, the products were subjected to agarose gel (1.5%)

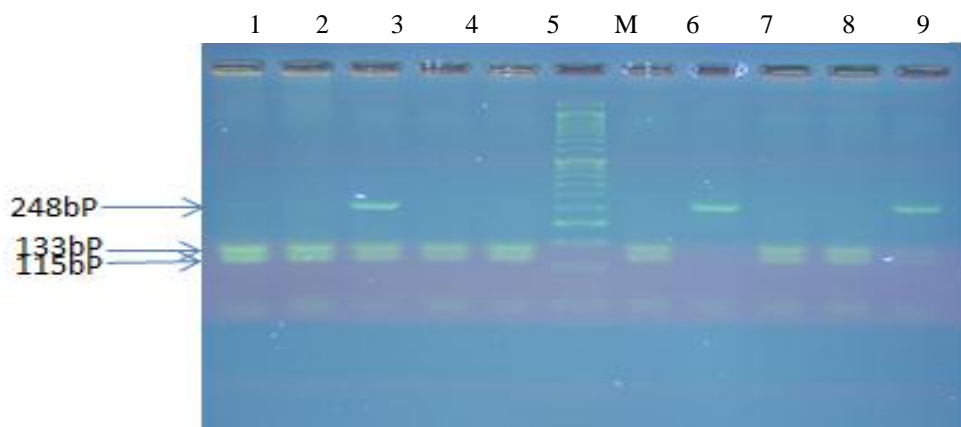
electrophoresis, followed by safe staining. The gels were photographed using an ultraviolet light transilluminator (Fig. 2).

**Statistical analysis of data**

The frequency differences of both polymorphisms were tested by Chi-square test between cases and controls, and odds ratio in 95% confidence intervals were determined using SPSS (ver. 16).



**Fig. 1.** Analysis of CYP1A1\*2A polymorphism by PCR-RFLP. Lane 5,10: CC homozygous (200 and140 bp); lane 4,9,11: TC heterozygous (340, 200 and 140 bp); lane 1,2,3,6,7: TT homozygous(340 bp).



**Fig. 2.** Analysis of CYP1A1\*2C polymorphism by PCR-RELP. Lane 1,2,4,5,6,8,9: AA homozygous (133 and 115 bp); lane 3: AG heterozygous (248, 133 and 115 bp); lane 7,10: GG homozygous (248 bp)

**Results**

The frequencies of the TT, TC and CC genotypes of CYP1A1\*2A polymorphism in cases and controls are shown in tables 1 and 2. The statistical analysis of the gene frequencies showed that CC genotype is associated with increased risk of male infertility (OR=5.4, 95% CI=1.12-26.04). The frequency of these polymorphisms is in the Hardy-Weinberg

equilibrium. The frequencies of CYP1A1\*2C genotypes in the patients (9.5, 84.8 and 5.7% for AA, AG and GG, genotypes respectively) were non-significantly different from those in controls (4.8, 94.2 and 1.0% for AA, AG and GG genotypes, respectively) (p=0.364 and p=0.164), as shown in tables 3 and 4.

**Table 1.** Genotype frequencies of CYP1A1\*2A polymorphisms in cases and the controls

	Genotypes	Cases N (%)	Control N (%)	OR	CI	P-value
CYP1A1*2A	TT	55 (52.4%)	66 (63.5%)	ref	--	--
	CC	9 (8.6%)	2 (1.9%)	5.4	(1.12-26.04)	0.036
	TC	41 (39.0%)	36 (34.6%)	1.37	(0.77-2.42)	0.285
	Sum	105	104			

OR= Odds ratio; CI= Confidence interval

**Table 2.** Allel frequencies of CYP1A1\*2A polymorphisms in cases and the controls

	Alleles	Cases N (%)	Control N (%)	OR	CI	P-value
CYP1A1*2A	T	151 (71/90%)	168 (80/77%)	0.61	(0.38-0.96)	0.034
	C	59 (28/1%)	40 (19/23%)			

OR= Odds ratio; CI= Confidence interval

**Table 3.** Genotype frequencies of CYP1A1\*2C polymorphisms in cases and the controls

	Genotypes	Cases N (%)	Control N (%)	OR	CI	P-value
CYP1A1*2C	AA	10 (9.5%)	5 (4.8%)	ref	--	--
	GG	6 (5.7%)	1 (1.0%)	3.00	(0.28-32.21)	0.364
	AG	89 (84.8%)	98 (94.2%)	0.45	(0.15-1.38)	0.164
	Sum	105	104			

OR= Odds ratio; CI= Confidence interval

**Table 4.** Allele frequencies of CYP1A1\*2C polymorphisms in cases and the controls

	Allele	Cases N (%)	Control N (%)	OR	CI	P-value
CYP1A1*2C	A	109 (51/90%)	108 (51/92%)	0.99	(0.68-1.46)	0.997
	G	101 (48/1%)	100 (48/08%)			

OR= Odds ratio; CI= Confidence interval

## Discussion

In this study, frequency of the two single nucleotide polymorphisms of CYP1A1 gene of infertile men, was tested. Genetic studies have shown that cytochrome P4501A1 (CYP1A1) plays a crucial role in phase I metabolism of polycyclic aromatic hydrocarbons, which have potential reproductive toxicity in men [6, 7]. Despite significant advancements in the diagnostic workup of infertile men, the cause in approximately 50% of cases remains unknown [18-20].

Reproductive functions in men may be impaired by many environmental, physiologic, and genetic factors [21-23]. The majority of the environmental factors are xenobiotics [6, 24]. Metabolic active xenobiotics exert adverse effects via covalent interactions between intermediate metabolites and cellular macromolecules such as DNA [25] and protein [26]. These compounds are not only metabolized by CYP1A1 but are also capable of inducing the activity of the enzyme. Apart from xenobiotic metabolism, CYP1A1 can participate in inactivation of testosterone [27] and, therefore, an increased enzyme activity could affect testicular function. To date, the impact of genetic variability to metabolize xenobiotic on male reproductive functions has not been extensively studied. The relation between environmental and genetic factors and infertility has not been shown clearly. In the present study, we determined the relation between these CYP1A1 polymorphisms, which are related to xenobiotic-metabolizing enzymes in nonobstructive azoospermia referring to

Yazd Reproductive Sciences Institute. Thus, the individuals carrying CYP1A1\*2A CC allele were found to have an increased risk of infertility. Since the original identification of the CYP1A1 polymorphisms, a number of studies have investigated the genetic effect of the polymorphisms on susceptibility to human complex diseases, such as various cancers [28, 29] polycystic ovary syndrome [30], chronic kidney disease [31], coronary artery disease [32] and systemic lupus erythematosus [33]. Association between polymorphisms of CYP1A1 and susceptibility to male infertility has also been reported in different populations with polymorphisms of CYP1A1 gene. Previous study found that individuals with CYP1A1 mutations have significantly increased risk for the development of idiopathic male infertility in Indian and Chinese populations [34, 35]. However Yarosh et al. revealed that CYP1A1 variants have no effect on the male infertility [36]. Recently, the important influence of estrogen in the development of male infertility has been acknowledged [36, 37]. It is well recognized that estrogens are metabolized by CYP1A1, as an estrogen-metabolizing gene, and converted into catecholestrogens 2-hydroxyestradiol and 4-hydroxyestradiol [38]. CYP1A1 is also involved in inactivation of xenobiotic metabolism, and activation of environmental toxins. There is a complex interaction circuit between CYP1A1, estrogen receptor alpha, and aryl hydrocarbon receptor with anti-estrogenic properties [39, 40]. CYP1A1 is

induced by diverse exogenous and endogenous chemicals through the aryl hydrocarbon receptor [41]. Moreover, CYP1A1 expression interacts with the aryl hydrocarbon receptor and estrogen receptor alpha expression [42]. CYP1A1 polymorphisms can alter the activity and expression of the enzyme [43, 44]. They can regulate the expression level of aryl hydrocarbon receptor and estrogen receptor alpha, resulting in male reproduction disorders. In addition, association of CYP1A1 and estrogen polymorphisms with impaired spermatogenesis implies that both genetic and environmental factors contribute to testicular dysfunction, which can lead to sperm damage, deformity, and eventually male infertility [45]. In the present study the frequency of the CYP1A1\*2A CC genotype in cases was higher than controls, indicating that the individuals carrying this allele have an increased risk of infertility independent of seminal parameters. Other studies also indicate that polymorphism in the gene CYP1A1 has been shown to be associated with male factor infertility [35,46,47]. These results suggest that genetic

polymorphisms of xenobiotic-metabolizing enzymes can play an important role in infertility. Therefore, CYP1A1 polymorphisms, in the standard semen analysis, might be taken as important parameters for prediction of fertility potential.

## Conclusions

With regard to the results of the association between a polymorphism of CYP1A1\*2A and azoospermia this single nucleotide polymorphism can be recognized as an effective agent in azoospermia, although their function is dependent on other genetic and environmental factors. Molecular studies are needed at other genetic levels to better understand its precise genetic role.

## Conflict of Interest

The authors declare that they have no competing interests.

## Acknowledgment

There is no acknowledgement to declare.

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