

Genetic variation of choline dehydrogenase gene in idiopathic male infertility

Received: December 15, 2013; Accepted: March 5, 2014

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ABSTRACT

Infertility can be caused by an unexplained reduction in semen quality in males who present as normal on physical examination and endocrine testing. There is some evidence that aberrant metabolism of micronutrients such as choline may play a causative role in male factor infertility. Choline is a crucial factor in the regulation of sperm membrane structure and motility, and this nutrient plays an important role in the maturing and fertilizing capacity of spermatozoa. In the present study, we explored the contribution of the choline dehydrogenase gene polymorphism located in the codon 78 (*CHDH* +432G>T), one of the basic enzymes of choline metabolism, to idiopathic male infertility. In this study, 50 infertile men and 50 fertile men of the Guilan population were selected. Genomic DNA was extracted from peripheral blood. Genotypes were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Statistical analysis was performed using MedCalc software (v12.1.4.0). A significant difference was observed between patients and healthy subjects in the distribution of G and T alleles. The prevalence of genotype frequencies of *CHDH* +432 GG, GT, and TT were 28%, 50%, and 22%, respectively, in patients, while in healthy subjects they were 52%, 36%, and 12%, respectively. In other words, there was a significant difference in the genotype distribution of *CHDH* +432G>T in patients compared with controls ($P<0.05$). This finding suggests a possible influence of this gene polymorphism on idiopathic male infertility.

Keywords: Choline dehydrogenase, gene polymorphism, infertility.

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Introduction

Infertility is described as the inability of couples to conceive a child after at least 12 months of regular, unprotected intercourse (1). Male factor accounts for 20% of infertility causes and has a contributory role in 30-40%, so male factor infertility is responsible for more than 50% of infertility difficulties (2). No recognizable reason is found in some cases of male infertility. This is called idiopathic male infertility. It is also defined as an unexplained reduction in semen quality in terms of sperm count, motility, and morphology in males who receive normal results in physical examination and endocrine testing (3). Infertility has genetic and non-genetic causes (2, 3). Recognizing and understanding how genetic abnormalities influence spermatogenesis and fertilization will improve the possibility of defeating male infertility. Furthermore, based on data showing associations among genetic variations, nutrient metabolism, and male factor infertility, effective male contraception may be promoted (4).

Though the association of whole nutritional condition with reproduction is well documented (5-7), the relationship between micronutrient metabolism and reproduction is understudied. There is some evidence that aberrant micronutrient metabolism may perform a causative role in male factor infertility. Dietary deficiencies of vitamins A, C, and E and trace metals such as zinc and selenium have also been found to be related with male infertility in animals and humans (4). Choline is a dietary component essential for structural integrity, and it has signaling roles for cell membranes. It affects cholinergic neurotransmission and lipid transport from the liver, and it is the main source of methyl groups via its metabolite. Choline is synthesized *de novo* or obtained from the diet (8, 9).

Choline dehydrogenase (CHDH) performs a significant role in choline metabolism by catalyzing the oxidation of choline to betaine in the inner membrane of mitochondria (10). Betaine acts as a methyl donor in the commutation of homocysteine to methionine and is an organic osmolyte consumed by cells for regulatory volume control (4, 11). Testicular betaine concentrations have been comprehended at 10 times the concentrations in the liver, the organ thought to be the primitive choline metabolism site. This detection suggests that betaine plays a major role in testicular function. Furthermore, testis, liver, and kidney tissues contain the highest CHDH activity; choline and betaine concentrations are most changed by *CHDH* gene deletion at these places (12). The importance of the role of the *CHDH* gene in the male reproductive system has been verified by studies of male *CHDH* knockout mice. The defective fertility of these mice was mainly due to decreased sperm motility, abnormal mitochondrial structure, inner membrane polarization, and reduced amounts of ATP (4).

A large number of variations in *CHDH* genes has been recognized in humans. Of these variants, *CHDH rs12676* (+432G>T) has been found to alter CHDH enzyme activity and thus can influence the metabolism of choline [11]. It is a non-synonymous SNP located in exon 3 of the *CHDH* gene. Occurrence of the *CHDH* +432T allele variant results in the substitution of arginine, a polar, hydrophilic amino acid with leucine, a hydrophobic amino acid (4, 13). Considering the above findings, the present study examined the potential association of the *CHDH* +432G>T polymorphism with idiopathic male infertility.

Materials and Methods

Subjects

The study population consisted of 50 patient subjects and 50 fertile men who had fathered at least 1 child as healthy control subjects from the Guilan population. No recognizable

reasons for the infertility of the idiopathic infertile men were found upon physical examination or endocrine laboratory testing. A written consent letter, detailed medical history, and general lifestyle description were obtained from all subjects.

Genomic DNA was extracted from peripheral blood leukocytes using a GPP-Solution kit (Gen Pajoohan, Iran) according to the manufacturer's instructions. 1.8% agarose gel electrophoresis was used to evaluate the quality of the extracted DNA.

Genotype analysis

Polymerase chain reaction was used to amplify a DNA fragment containing the *CHDH* +432G>T polymorphism, a non-synonymous SNP located in exon 3. The primers used for amplification were *CHDH* F: 5'-ATACCCTGGATATGCGGAGTT-3' and *CHDH* R: 5'-GCACCA GTTGTACCTGTCG -3'. Each 50 µl PCR reaction contained 60 ng of genomic DNA. The PCR conditions were denaturation at 95°C for 5 min, 30 cycles at 95°C for 1 min, 50.8°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 5 min. The 429bp PCR product was treated with restriction enzyme endonuclease *BssHII* (Fermantas, German) for 4 h at 37°C. The wild-type G allele was digested into two fragments, 342 and 87 bp, but there was no digestion site on the mutant T allele. The digested PCR products were separated on 3% agarose gel by electrophoresis and visualized by exposure to ultraviolet light after ethidium bromide staining. All reactions were run in duplicate with negative and positive controls and blanks. The resulting genotypes for *CHDH* (G/T) and polymorphic sites were characterized as GG/GT/TT.

Statistical analysis

Statistical analysis of the differences between allele and genotype frequencies was performed using the chi-square test (χ^2) and Odd Ratio (OR). *P* values less than 0.05 were considered statistically significant. All analyses were carried out using MedCalc software (v12.1.4.0).

Results

Study population

The subjects consisted of 50 infertile and 50 fertile men. The age of the infertile men ranged from 26 to 45 years, and the average was 35.22 years. The age of controls ranged from 27 to 41 years, and the average was 33.85 years. General characteristics of the study population are shown in Table 1.

Based on World Health Organization (WHO) criteria, standard semen analyses were performed for the infertile patients. The results revealed a range of sperm abnormalities in some patients. As shown in Table 2, the lowest and highest semen abnormalities were teratospermia and asthenospermia, respectively.

Genotype analysis

To investigate genotypic and allelic distribution of the *CHDH* +432G>T polymorphism in the studied population, the PCR-RFLP method was used. The 429 bp DNA fragment was amplified by PCR reactions containing the single nucleotide polymorphism mentioned above and digested with *BssHII*. Digested products (342 bp and 87 bp) were separated on 3% agarose gel. Banding patterns on agarose gel based on the type of genotypes GG/GT/TT were different (Figure 1).

Table 1. Characteristics of the studied population.

Feature	Cases, n (%)	Controls, n (%)	P value
Smoker	10 (20)	4 (8)	ns
Alcoholism	4 (8)	2 (4)	ns
Drug addicted	3 (6)	1 (2)	ns
Family background of infertility	5 (10)	0	ns
None of above	28 (56)	43 (86)	ns

Abbreviations: Data shown as number (n) and percentage (%); ns, not significant

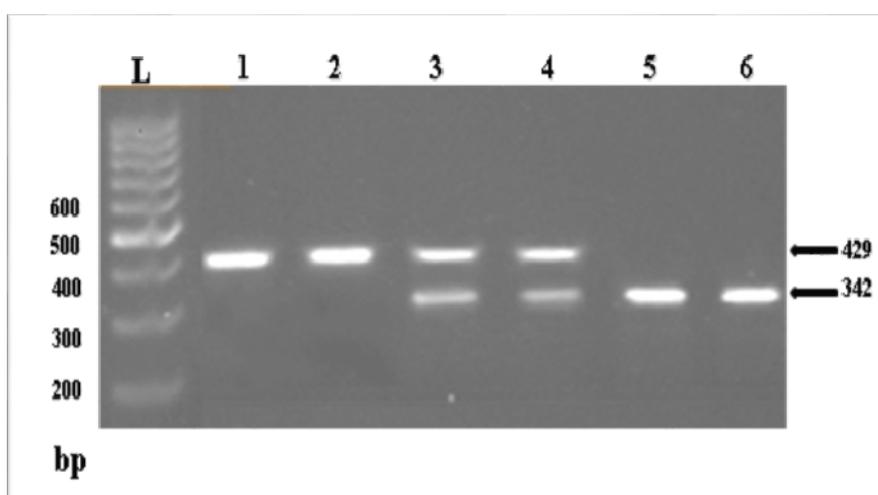


Figure 1. PCR-RFLP assay (3% agarose gel electrophoresis) of CHDH gene after restriction of the polymorphic region with *Bss*HI restriction enzyme: Lane 1, 2 – TT genotype; Lane 3, 4, – GT genotype; Lane 5, 6 – GG genotype. 429 and 342 bp fragments visible and marked with arrows but the 87 bp fragment was not visible. Lane L represents a 100 bp ladder.

Table 2. Semen analysis in infertile men*.

Semen variable	N (%)
Oligospermia	9 (18%)
Asthenospermia	11 (22%)
Teratospermia	6 (12%)
Oligoasthenospermia	10 (20%)
Normospermia	14 (28%)

*The standard semen analysis according to World Health Organization (WHO) in infertile patients was performed. Data shown are as number n and percentage (%).

Table 3. The genotypic and allelic distribution of the *CHDH* +432G>T polymorphism in control and infertile subjects.

Polymorphism	Genotypes/ alleles	Cases, n (%)	Controls, n (%)	OR	95% CI	P-value
<i>CHDH</i> +432G>T	GG	14 (28)	26 (52)	1.0	Reference	
	GT	25 (50)	18 (36)	2.57	1.06-6.27	0.03
	TT	11 (22)	6 (12)	3.40	1.03-11.17	0.04
	G	53 (53)	70 (70)	1.0	Reference	
	T	47 (47)	30 (30)	2.06	1.15 - 3.69	0.01

Abbreviations: CHDH, choline dehydrogenase; OR, Odds ratio, Data shown as number (n) and percentage (%).

Genotype and allele frequencies were different between fertile and infertile men. Allele G and T frequencies in patients were 53% and 47% and in healthy subjects were 70% and 30%, respectively. Significant differences between patients and controls were observed ($\chi^2=5.40$, $P<0.05$). Thus the presence of the T allele is considered to be a risk factor (OR=2.06, $P<0.05$) for infertility. For *CHDH* +432G>T, 28% of infertile men were GG, 50% were GT, and 22% were TT (Table 3). Among controls, 52% were GG, 36% were GT, and 12% were TT. Therefore, a significant difference in the genotype distribution of *CHDH* +432G>T was observed in patients compared with controls ($\chi^2=6.21$, $P<0.05$). The results suggest that the GT and TT genotypes are likely to increase the risk of infertility in men and are therefore considered to be risk factors.

Discussion

Nearly 13-15% of couples around the world are affected by infertility (3). Up to half of these cases occur from male factor infertility. Unknown genetic disorders, such as chromosomal deletions, translocations, and single nucleotide polymorphisms (SNPs), may be the main reason of many cases of idiopathic male infertility (4). Choline is a major source of methyl groups in the human

diet (14). Choline and its metabolites play necessary roles in the constitution of the methyl donor S-adenosylmethionine, which is involved in more than 80 biological methylation reactions containing the methylation of proteins, DNA, and RNA. About 56% of sperm plasma membrane phospholipids are formed from choline and ethanolamine phosphoglycerides (15). Additionally, glycerophosphorylcholine and choline, synthesized by the epithelial cells of the epididymis, are needed for the maturation of spermatozoa (16). This information supports the possibly significant role of choline metabolism in spermatogenesis. Changes in the enzymes participating in the metabolism of choline, such as choline dehydrogenase, can likely affect the spermatogenesis process due to gene sequence variations and single nucleotide polymorphisms (11).

The current study examined the allelic and genotypic distribution of the *CHDH* +432G>T polymorphism in fertile and infertile men and its association with infertility. Data showed that the polymorphism described above may influence human spermatogenesis and, consequently, fertility. The genotypic and allelic distributions of the *CHDH* +432G>T polymorphism presented notable differences

between fertile and infertile men. The *CHDH* 432G/T and 432T/T genotypes were associated with a higher risk factor compared with the *CHDH* 432G/G genotype in infertile men.

In our study, analysis of the *CHDH* +432G>T polymorphism revealed a noteworthy association of the *CHDH* +432T allele with male infertility. It has been previously reported that the T allele has a negative effect on human sperm concentrations (10). Even among normozoospermic men, the *CHDH* 432T/T genotype carriers have the lowest sperm concentration (10, 11). Johnson *et al.* showed that *CHDH* deletion causes diminished sperm motility, probably due to changes in mitochondrial morphology, function, and ATP content (4). *CHDH* converts choline to betaine aldehyde, which is then oxidized to betaine via betaine aldehyde dehydrogenase, a methyl donor for homocysteine. Prior studies have shown that carriers of the minor *CHDH* 432T allele present with an increased susceptibility to expanding organ dysfunction on a low choline diet, such as steatosis and muscle cell damage (13) as well as increased risk of breast cancer (17). The *CHDH* +432G>T single nucleotide polymorphism generates an amino acid replacement that could change enzyme activity and, consequently, choline metabolism. However, additional studies are needed to determine how *CHDH* 432 alleles influence the *CHDH* gene or protein activity. Oxidation of one choline molecule to betaine eventuates in the production of 5 ATP molecules by the mitochondrial electron transport chain (18). It is possible that the betaine molecule itself performs a major role in maintaining testicular and sperm cell function and especially spermatogenic ATP contents (12). Betaine is an organic osmolyte used by cells

for guarding during times of osmotic stress (19). Sperm mature as they move from the lumen of the testis and several regions of the epididymis and accumulate molecules found within the epididymal environment including organic osmolytes (20). The epididymal environment is relatively hyperosmotic in comparison with the osmolality of unliquefied whole semen and the fluid in the female reproductive tract (21, 22), indicating that epididymal sperm withstand an osmotic challenge within the male urethra. An inability to regulate volume in response to various osmotic environments would render sperm sensitive to swelling which could impair motility (22). Moreover, the reduced testicular *CHDH* activity, the almost undetectable testicular choline and betaine concentrations in the case of *CHDH* gene deletion, and the asthenospermic phenotype of the male *CHDH* knockout mice (12) enhance the importance of *CHDH* in the male reproductive system. Overall, this evidence demonstrates that altered *CHDH* activity due to the *CHDH* +432G>T genotype may be a main cause of idiopathic male factor infertility. This is an especially interesting finding, because deficits in *CHDH* function may be overcome by dietary supplementation with betaine. In addition, as noted above, sperm motility and ATP content were improved in male *CHDH* knockout mice ingesting betaine-supplemented drinking water (12).

In this study, a notable association of the *CHDH* 432T allele with idiopathic male infertility was observed. Considering the above findings, we hypothesize that the *CHDH* 432T allele may have a negative influence on *CHDH* activity, causing diminished testicular betaine concentrations, lower testicular ATP content, reduced sperm concentrations and, consequently, male

infertility. Some data suggest that *CHDH* +432G>T is a functional SNP, or that it is a tag SNP (12) that marks a functional haplotype of the *CHDH* gene, although additional studies that investigate the association of the *CHDH* 432T allele with testicular *CHDH* activity and betaine levels are needed to clarify the association of this allele with impaired spermatogenesis and fertility.

To the best of our knowledge, this study is the first to show that the association of the *CHDH* +432G>T polymorphism with idiopathic male infertility is likely caused by changes in testicular choline dehydrogenase transcripts or action. Even though the number of cases enrolled in this study may limit the

potency of our conclusion, our findings are nevertheless indicative of the association of the *CHDH* enzyme with male fertility. The confirmation of our preliminary results in larger groups of infertile men, the simultaneous measurement of testicular *CHDH* levels, transcripts, and *CHDH* activity, and the evaluation of sperm nuclear maturation may give evidence for the contribution of this gene to the efficiency of spermatogenesis and thus fertility.

Acknowledgments

The authors would like to extend special appreciation to all participants in this study for their contribution. There is no conflict of interest in this research.

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