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Association of N-acetyltransferase-2 and glutathione S-transferase polymorphisms with idiopathic male infertility in Vietnam male subjects

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ABSTRACT

N-acetyltransferase-2 (NAT2) and Glutathione S-transferases (GSTs) are phase-II xenobiotic metabolizing enzymes participating in detoxification of toxic arylamines, aromatic amines, hydrazines and reactive oxygen species (ROS), which are produced under oxidative and electrophile stresses. The purpose of this research was to investigate whether two common single-nucleotide polymorphisms (SNP) of NAT2 (rs1799929, rs1799930) and GSTP1 (rs1138272, rs1695) associated with susceptibility to idiopathic male infertility. A total 300 DNA samples (150 infertile patients and 150 healthy control) were genotyped for the polymorphisms by ARMS - PCR. We revealed a significant association between the NAT2 variant genotypes (CT + TT (rs1799929), (OR: 3.74; $p < 0.001$)) and (GA + AA (rs1799930), (OR: 3.75; $p < 0.001$)) or GSTP1 variant genotypes (GA + AA (rs1695), (OR: 5.11; $p < 0,001$)) and (CT + TT (rs1138272), (OR: 7.42; $p < 0,001$) with idiopathic infertility risk. Our findings rate the effect of single-nucleotide polymorphisms of GSTP1 and/or NAT2 in modulation of the risk of male infertility in subjects from Vietnam. This pilot study is the first (as far as we know) to reveal that polymorphisms of NAT2 (rs1799929, rs1799930) and GSTP1 (rs1138272, rs1695) are some novel genetic markers for susceptibility to idiopathic male infertility.

1. Introduction

Infertility is a worldwide reproductive health problem affecting approximately 12%–15% of couples [1]. In about half of the cases, poor semen quality is a major cause [2]. Male infertility represents a typicalexample of complex disease with a substantial genetic basis [3]. Its etiology and pathogenic mechanism remain unknown in about 30% of cases; this phenomenon is known as *idiopathic infertility* [4]. Male germ cells are highly susceptible to stress; therefore, protection from oxidative stress is an important way to boost fertility. Xenobiotics make up the majority of environmental factors. They include naturally occurring compounds, drugs, environmental agents, and carcinogens. Adverse effects of xenobiotics are exerted via covalent interactions between intermediate metabolites and genetic components or proteins and their related metabolites [5].

Glutathione S-transferases (GSTs) or N-acetyltransferases (NATs) are the members of superfamily of ubiquitous multifunctional enzymes that play a key role in phase-II cellular detoxification and bioactivation reactions, and are generally considered to be "antioxidant" enzymes [6]. They are widely expressed in mammalian tissues and have broad substrate specificity. Individuals lacking GST-M1, GST-T1 and GST-P1 genes have a higher incidence of male infertility, bladder, breast, colorectal, head/neck and lung cancer [7].

Genetic-based alterations in GSTs' activity might alter their ability to detoxify potentially damaging agents, and hence, increase the risk of disease development. SNP of the gene encoding GSTM1, GSTT1 or GSTP1 polymorphisms (Ile105Val and Ala114Val) can affect the binding affinity of these enzymes, and/or reduce or eliminate cellular GST activity. GSTP1 plays a critical role in biotransformation and inactivation of toxic and carcinogenetic electrophiles, especially those in cigarette smoke, and also inhibits apoptosis and promotes cellular proliferation through interacting with Jun N-terminal kinase (JNK) pathway. Polymorphisms (Ile105Val and Ala114Val) of GSTP1 gene significantly alter the activity and heat stability of this enzyme decreasing its ability to detoxify environmental mutagens and protect against oxidative DNA damage [3]. Tirumala Vani et al. [8] have identified an association between the GSTM1 null genotype and idiopathic male infertility. Olshan et al. [9] have reported that the GSTT1 non-null genotype is associated with reduced sperm concentration and count. Safarinejad et al. [10] have revealed an increased risk of GSTM1 and/or GSTT1 null genotypes for developing infertility with a protective effect conferred by the GSTP1 variant genotypes. Wu et al. [11]

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Table 1
Primers for the multiplex tetra-primer ARMS-PCR for GSTP1 and NAT2 mutations.

SNP ID	Gene	Outer primer (5′-3′)	Inner primer (5′-3′)	bp
rs1138272	GSTP1	5′-CAGGTGTCAGGTGA GCTCTGAGCACC-3′ 5′- ATAAGGGTGCAGGTTG TGTCTTGTCCCA-3′	5′-CGTGGAGGACCTCCGC TGCAAATC CA-3′ 5′-GCTCACATAGTTGGTGT AGATGAGGGATAC-3′	392
rs1695	GSTP1	5′-AGGTTACGTAGTTTGCC CAAGGTC-3′ 5′-CGTTACTTGGCTGGTTG ATGTCC-3′	5′-GAGGACCTCCGCTGCAA ATTCG-3′ 5′- CATAGTTGGTGTAGATG AGGGAGCT-3′	360
rs1799929	NAT2	5′-CAGGTGCCTTGCATTT TCT-3′ 5′-GATGAAGCCCACCAA ACAGT-3′	5′- CCAATAAAAGTAGAAG CGA-3′ 5′- CTCTTCCAGGACCTCCA-3′	375
rs1799930	NAT2	5′- AAAGAATTTCTTAATT CTCATCTCCTG-3′ 5′-AAAATGATGTGGTTATA AATGAAGATG-3′	5′- ACCACAGATCGAAGGT CGGAATATAC-3′ 5′- TTATTTACGCTTGAACC TC-3′	401

have found that the GSTT1 null genotype is a predisposing risk factor for sporadic idiopathic azoospermia or oligospermia. Tang et al. [12] have revealed that GSTM1 and GSTT1 null genotypes may predispose sperm to increase oxidative damage in infertile men with varicoceles while the GSTP1 allelic variations do not display any differences between the experimental and control groups.

Arylamine N-acetyltransferases (NAT1 and NAT2) are the polymorphic enzymes responsible for the acetylator phenotype. Individual differences in NAT metabolic capacity are caused by allelic variations of the NAT gene, which are determined by the pattern of single-nucleotide polymorphisms, that results in slow (homozygous carriers for low activity alleles), intermediate or rapid (carriers of one or more high activity alleles) acetylator phenotypes. Rapid and slow acetylations are known to be the predisposing factors for individual's sensitivity to toxicity through exposure to large number of arylamines [13]. The frequency of specific mutations within the NAT loci depends on racial and ethnic origin of men. Phenotype analyses have revealed an association between slow NAT2 acetylation genotype and the risk of lung, colon, liver or bladder cancer [14–16]. At the same time, rapid acetylator phenotype is associated with a higher level of DNA rupture in subjects after two days of meat diet [17]. Lammer et al. have found evidence to prove that there is connection between NAT1 polymorphism, lack of maternal multivitamin consumption and presence of birth defects (cleft lip) [18]. The results obtained in another study have showed an increased risk of prostate cancer for subjects with NAT1 and NAT2 slow acetylation genotypes [19].

The SNP 481C > T (rs1799929) and 590G > A (rs1799930) are known to be the most common and functionally important *NAT2* genetic variations. It has been found that C > T nucleotide substitution at position 481 does not alter the amino acid chain of the enzyme (known as a synonymous SNP, L161L), whereas the G > A nucleotide substitution at position 590 changes arginine to glutamine at codon 197 (R197Q). Thesepolymorphisms are associated with disequilibrium and cause decreased activity of NAT2 enzyme determining a low acetylator phenotype in men [20].

In this study, we have conducted an analysis to illustrate the possible role of GSTP1 and NAT2 gene in male infertility.

2. Materials and methods

2.1. Subjects

We have carried out a case-control study, which involved 150 infertile patients with abnormal spermiogram (80 subjects with non-obstructive azoospermia and 70 – with oligospermia), who have been attending the Hanoi Medical Hospital (Hanoi, Viet Nam) from June 2016 to May 2017. All men had an infertility history for at least 1.5 years without indication of hormonal, medical, or surgical causes; their spouses had a normal gynecological assessment. We excluded patients that involved histories of epididymo-orchitis, prostatitis, genital trauma and testicular tumors; such genital diseases as cryptorchidism, congenital bilateral absence of the vas deferens or varicocele; seminal infections; diabetes; Y-chromosome microdeletions, cytogenetic, or karyotype abnormalities; drug, alcohol, or substance abuse; and tobacco use. Semen analysis was performed according to World Health Organization recommendations, 2010. We have selected 150 fertile men of comparable age, who had fathered at least one child without assisted reproductive technologies, for the control group. All participants were informed about the study according to the protocol that was approved by the center of genetics counselling at the Hanoi Medical University (Hanoi, Viet Nam); all subjects gave their written consents.

2.2. ARMS - PCR - analysis

Key elements of optimizing tetra primer ARMS PCR are determining the ratio of outer and inner primers, as well as the annealing temperature, while minimizing unspecific band. These factors were put into action by using gradient PCR system (different annealing temperatures in one go) along with different outer and inner primer ratios in the optimizing process. Genomic DNA was extracted from peripheral venous blood with a DNA Express Purification Kit (Lytech, Russsia) according to the manufacturer's instructions. Spectroscopy was performed to quantify the amount of extracted genomic DNA. DNA samples were stored at - 20 °C. Inner primers are SNP-specific.

The ARMS (amplification-refractory mutation system) was used to identify four alleles of *GSTP1* and four alleles of *NAT2* with four different sets of primers (Table 1). The outer primers have the same function as primer in conventional PCR – isolating and amplifying DNA sequence of interest, while the inner primer is used to detect allelic variation.

PCR was performed for each DNA sample in 50 μl reaction volume containing 5 μl (50–100 ng) of genomic DNA; each tube (normal allele and pathology allele) contained 17.5 μl of PCR buffer, 2,5 μl of primer mix (normal allele or pathology allele), 0.2 μl of Taq DNA polymerase and 25 μl of mineral oil (Lytech, Russia). PCR was performed with initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min, elongation at 72 °C for 2 min, and a final extension at 72 °C for 10 min. The 151–218 bp fragments were amplified.

PCR product was analyzed on a 3% agarose gel prepared in 1 × TBE buffer containing OneRed nucleic acid gel stain (Biotium, America) at 120 V for 20 min at room temperature.