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ARTICLE

Synergism between the *N*-acetyltransferase 2 gene and oxidant exposure increases the risk of idiopathic male infertility

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Abstract *N*-acetyltransferase (NAT2) is a phase-II xenobiotic-metabolizing enzyme participating in the detoxification of toxic arylamines, aromatic amines and hydrazines. The present study was designed to investigate whether two common single-nucleotide polymorphisms (SNP) of the *NAT2* gene (481C>T, rs1799929; 590G>A, rs1799930) are associated with susceptibility to idiopathic male infertility and to assess if the risk is modified by oxidant and antioxidant exposures. A total 430 DNA samples (203 infertile patients and 227 fertile men) were genotyped for the polymorphisms by PCR and restriction fragment length polymorphism. No association was found between the *NAT2* polymorphisms and idiopathic male infertility. However, gene–environment interaction analysis revealed that a low-acetylation genotype, 590GA, was significantly associated with increased disease risk in men who had environmental risk factors such as cigarette smoking (OR 1.71, 95% CI 1.02–2.87, $P = 0.042$), alcohol abuse (OR 2.14, 95% CI 1.08–4.27, $P = 0.029$) and low fruit/vegetable intake (OR 1.68, 95% CI 1.01–2.79, $P = 0.04$). This pilot study found, as far as is known for the first time, that the polymorphism 590G>A of *NAT2* is a novel genetic marker for susceptibility to idiopathic male infertility, but the risk is potentiated by exposure to various environmental oxidants.

KEYWORDS: alcohol abuse, cigarette smoking, fruit and vegetable intake, gene–environment interaction, idiopathic male infertility, *N*-acetyltransferase 2 (NAT2)

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Introduction

Male infertility is widespread and affects approximately 10–15% of the global male adult population. Idiopathic infertility (also known as an unexplained infertility) is found in 40–75% of infertile men (Faasse and Niederberger, 2012; Rowe et al., 2000). A progressive increase in prevalence of male infertility and also a decrease in sperm quality have been found worldwide (Carlsen et al., 1992; Jouannet et al., 2001; Kunstmann et al., 1995; Winters and Walsh, 2014). Despite considerable research efforts, the aetiology and the mechanisms underlying male infertility are still the subject of debate (Singh and Jaiswal, 2011). At the same time, a growing body of epidemiological studies clearly suggests that a male infertility epidemic in the world can be related to global changes in the environment over the last decades (Auger et al., 2001; Han et al., 2011; Hansen et al., 2010; Jørgensen et al., 2006). It has been proved that many chemical agents of the environment are responsible for adverse effects on male reproductive organs and spermatogenesis, and are likely to be the leading causes of male infertility in the modern world (Gaspari et al., 2003; Hansen et al., 2010; Horak et al., 2003; Jurewicz et al., 2009; Mendiola et al., 2008).

It is well known that interindividual differences in the ability to activate and detoxify chemical substances of the environment are attributed to the genetic variability of biotransformation or xenobiotic-metabolizing enzymes (Nebert et al., 1996, 2013). Polymorphic genes for xenobiotic-metabolizing enzymes are considered to be important modifiers of susceptibility to male infertility (Aydos et al., 2009; Rubes et al., 2010; Schuppe et al., 2000; Yarosh et al., 2013). Many studies have observed that functional polymorphisms in genes such as *CYP1A1*, *GSTM1* and *GSTT1* are associated with the risk of idiopathic male infertility (Aydos et al., 2009; Jaiswal et al., 2012; Polonikov et al., 2010; Safarinejad et al., 2010).

Arylamine *N*-acetyltransferase 2 (NAT2) is one of the phase-II xenobiotic-metabolizing enzymes that detoxify a number of environment chemicals such as arylamines, hydrazines, aromatic and heterocyclic amines into their intermediates through reactions of *N*-acetylation and *O*-acetylation (Blum et al., 1991; Hein et al., 2000). Human *NAT2* is expressed in the male reproduction system, including testicular tissues, prostate, genital ducts and exocrine glands where the enzyme may have a protective role against chemicals responsible for male urogenital diseases (Husain et al., 2007; Wu et al., 2013). In particular, the enzyme acetylates benzidine and 2-naphthylamine (harmful aromatic amines of cigarette smoke), 2-aminofluoren (a dye widely used in industry), hydrazine-containing drugs (isoniazid, simendan) and heterocyclic amines (chemicals in meat cooked at high temperatures) are derived from their metabolic activation by cytochrome P450 1A2 (Guengerich, 2000; Hein, 2002; Hein et al., 2000). NAT2 has also been found to activate carcinogens, thereby producing unstable electrophiles that may induce point mutations in DNA (Hein et al., 1993).

NAT2 is a highly polymorphic intronless gene having more than 60 alleles (Vatsis et al., 1995). Polymorphisms in *NAT2* are responsible for the slow (homozygous carriers for low-activity alleles) and rapid (carriers of one or more high-activity alleles) acetylator phenotypes, each occurring with a frequency of about 50% in European and African populations and slightly less frequently in other racial/ethnical groups of the world (Hein et al., 2000). The SNP 481C>T (rs1799929) and 590G>A (rs1799930) are located and are known to be the most common and functionally important genetic variations in *NAT2* (Hein, 2002; Hein et al., 2000). It has been found that a C > T nucleotide substitution at position 481 does not alter the amino acid chain of the enzyme (known as a synonymous SNP, L161L), whereas a G > A substitution at nucleotide 590 causes an amino acid change from arginine to glutamine at codon 197 (R197Q; Cascorbi et al., 1995). These polymorphisms are in linkage disequilibrium and result in decreased activity of NAT2 enzyme, determining a low acetylator phenotype in men (Cascorbi et al., 1995).

Acetylator phenotype and related genetic variants of *NAT2* have been associated with the risk of various types of cancers (Cui et al., 2011; Gong et al., 2011a; Ma et al., 2013; Ying et al., 2011; Zheng et al., 2012; Zhong et al., 2010). In recent years, much attention of andrologists has been given to investigating the roles of *NAT2* in the development of cancers affecting the male reproductive system (Agúndez, 2008; Gong et al., 2011b). As far as is known, no studies have been designed to investigate the associations between *NAT2* polymorphisms and the risk of idiopathic infertility in men.

The purpose of this pilot study was to investigate whether two common SNP of *NAT2*, 481C>T and 590G>A, are associated with susceptibility to idiopathic male infertility. It was also important to assess whether oxidant and antioxidant exposures of the environment modify the relationship between *NAT2* polymorphisms and disease risk.

Materials and methods

Study population and diagnosis

The study was performed in keeping with the principles of the Helsinki Declaration. Each participant gave informed consent before enrollment in the study. The study protocol was approved by the Ethical Review Committee of Kursk State Medical University (reference number 6–07, approved 4 October 2007).

A total 430 unrelated Russian men were recruited for the study from the Family Planning and Reproductive Health Clinic of Kursk Regional Perinatal Centre over a period from 2006 to 2008. All eligible patients and controls matching the inclusion/exclusion criteria were given the opportunity to be enrolled in the study groups. The case group comprised 203 men with diagnosis of idiopathic male infertility. Criteria for inclusion in the case group were infertility for at least 12 months of regular unprotected intercourse with at least two repeated findings of abnormal sperm parameters and negative mixed agglutination reaction test. All recognizable causes of male infertility (varicocele, hypogonadotrophic hypogonadism, abnormal karyotypes, microdeletions of chromosome Y, abnormal sexual and ejaculatory functions and seminal tract obstruction) were excluded by a experienced andrologist, endocrinologist, geneticist and laboratory assistants. Only males with normal female partners of ovulatory age were included in the study; female infertility factor was excluded by experienced gynaecologists after thorough instrumental and laboratory investigations, including hysterosalpingography and/or hysteroscopy, biochemical tests for serum hormone profiles, karyotyping and molecular analysis for urogenital infections. The control group included 227