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Gene-gene and gene-environment interactions on risk of male infertility: Focus on the metabolites

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ABSTRACT

Infertility affects about 17% couples, and males contribute to half of the cases. Compared with independent effects of genetic and environmental factors, interactions between them help in the understanding of the susceptibility to male infertility. Thus, we genotyped 25 polymorphisms, measured 16 urinary chemical concentrations and explored interactions between gene-gene and gene-environment in 1039 Han Chinese using metabolomic analysis. We first observed that *GSTT1* might interact with *GSTM1* ($P_{inter} = 6.33 \times 10^{-8}$). Furthermore, an interaction between *GSTM1* and 4-*n*-octylphenol (4-*n*-OP) was identified ($P_{inter} = 7.00 \times 10^{-3}$), as well as a 2-order interaction among *GSTT1*, *GSTM1* and 4-*n*-OP ($P_{inter} = 0.04$). Subjects with *GSTT1*-present and *GSTM1*-null genotypes were susceptible to male infertility when exposed to 4-*n*-OP (OR = 14.05, 95% CI = 4.78–60.20, $P = 2.34 \times 10^{-5}$). Most metabolites identified were involved in the tricarboxylic acid cycle. In conclusion, it is a novel study of the interaction on male infertility from the aspect of metabolomics.

1. Introduction

Infertility is a reproductive disease defined by the failure to conceive after 12-month regular unprotected sexual intercourse (Zegers-Hochschild et al., 2009). In China, a latest prospective follow-up study claimed that the incidence of 12-month infertility was 13.6% among all eligible couples (Meng et al., 2015). About half of the cases link to a male factor among infertile couples (Tian et al., 2014). Except those who can be treated by assisted reproductive technology, there are still men incapable of fertilization. Thus, it is worthy of identifying factors potentially correlated with male infertility to prevent and control such a problem.

Spermatogenesis is a complex process in the area of male reproduction, and it can be influenced by many factors such as infection, sex hormone imbalance, tobacco smoking, and alcohol use (Diao et al., 2014; La Maestra et al., 2014; Wang et al., 2009). In the past few years, around the topic of genetic causes, microdeletions and copy number variations in the Y chromosome have been proved to be connected with spermatogenesis failure (Foresta et al., 2001; Lo Giacco et al., 2014). Furthermore, both traditional targeted approach and high-throughput genome-wide association study (GWAS) strategy have successfully identified some susceptibility loci linking with non-obstructive azoospermia (Hu et al., 2014; Hu et al., 2012). However, such variants merely accounts for a part of conceivable reasons, and there have been limited studies, for the purpose of validation, successfully created animal models of male infertility using single nucleotide polymorphisms (SNPs) identified by GWA studies.

To decipher the unknown part of male infertility, researchers have also observed a contribution of the environment. In the scope of daily life and occupational health, heavy metal is a toxicant reported to associate with abnormal semen quality (Meeker et al., 2008; Zeng et al., 2015). And our previous studies have also assessed the risks of some environmental endocrine disrupting chemicals (EDCs), like polycyclic aromatic hydrocarbons, phenols and phytoestrogens that are related to the male infertility (Chen et al., 2014; Xia et al., 2013; Xu et al., 2014). Nevertheless, the role of environmental factors in the development of complex diseases is restricted as the genetics. A combination, or the so called interaction, is considered to be a prospective direction (Koehl, 2015; Manuck and McCaffery, 2014).

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Gene-gene and gene-environment interactions are usually applicable to estimate the synergistic or antagonistic effects between both factors. And interactions have been explored to play essential roles in cancers, heart diseases, diabetes, etc. (Fedeles et al., 2011; Langenberg et al., 2014). Hauser et al. (2005) also identified a joint effect related to sperm motility in a study of 303 people. Given the limited and specific subfertile study population, the power of study like that is not convincing. For the purpose of supplying the lack of investigations on male infertility, we recruited 420 fertile men as well as 619 infertile cases in Han Chinese, examined gene-gene-environment interactions between two groups, and tried to deepen the comprehension of such interactions with a metabolomic analysis in the present study.

2. Methods

2.1. Study population and sample collection

Male infertility was characterized with at least two-year infertility history in our research. 1713 infertile men and 710 fertile men were invited to participate in the study consecutively from affiliated hospitals of Nanjing Medical University between 2005 and 2010. Of those, 1182 men (case = 700, control = 482) consented to participate and signed an informed consent. A questionnaire was utilized to collect characteristic information. All subjects claimed that their life styles and exposures had not been changed for several months before sample collection. After completion of the questionnaire, the examiner measured the height and weight of each participant. Males with abnormal sexual and ejaculatory functions, immune infertility, medical history of risk factors for infertility and other known causes related to male infertility, such as genetic disease, were excluded from the study. Although subjects were requested to donate a urine sample for the measurement of urinary EDCs concentrations, a 5 mL peripheral blood for genotyping and an ejaculate for semen analysis, some of them left no blood or semen. Hence, we included 1039 subjects (case = 619, control = 420) in this study at last. Among these 619 infertile cases, we collected 391 semen samples which were obtained in private by masturbation, and routine semen analyses were carried out according to World Health Organization guidelines. Standard measurements we used were sperm concentration (normal ≥ 15×10^6 spermatozoa/mL), progressive motility (normal ≥ 32%) and sperm morphology (normal ≥ 14%). Besides semen samples, we also kept urine samples at −20 °C while blood samples at −80 °C until measurement. The study protocol and informed consent were approved by the Institutional Review Board of Nanjing Medical University prior to the study. All activities involved in this study were done under full compliance with government policies and the Helsinki Declaration.

2.2. Measurement of urinary EDC concentrations

EDCs included in the study were bisphenol A (BPA), pentachlorophenol (PCP), benzophenone-3 (BP-3), triclosan (TCS), 4-*tert*-octylphenol (4-*t*-OP), 4-*n*-octylphenol (4-*n*-OP), 4-*n*-nonylphenol (4-*n*-NP), 2,3,4-trichlorophenol (2,3,4-TCP), 2,4,5-trichlorophenol (2,4,5-TCP), secoisolariciresinol (SEC), enterodiol (END), enterolactone (ENL), naringenin (NAR), genistein (GEN), daidzein (DAI) and equol (EQU). We measured urinary phenol and phytoestrogen concentrations using ultra high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) and gas chromatography–mass spectrometry (GC–MS) with sensitive methods as previously described (Chen et al., 2013; Xia et al., 2013). Limits of determination (LOD) were 0.36 ng/mL (BPA), 0.41 ng/mL (PCP), 0.04 ng/mL (BP-3), 0.9 ng/mL (TCS), 0.34 ng/mL (4-*t*-OP), 0.02 ng/mL (4-*n*-OP), 0.02 ng/mL (4-*n*-NP), 0.28 ng/mL (2,3,4-TCP), 0.15 ng/mL (2,4,5-TCP), 0.09 ng/mL (SEC), 0.04 ng/mL (END), 0.04 ng/mL (ENL), 0.02 ng/mL (NAR), 0.09 ng/mL (GEN), 0.03 ng/mL (DAI) and 0.12 ng/mL (EQU). Urinary creatinine (CR) concentration was analyzed with an automated chemistry analyzer (7020 Hitachi, Tokyo, Japan) for correcting the variation caused by fluctuated urine concentration and dilution.

2.3. Genotype identification

We identified variants in phase I and phase II enzyme genes in relation to the metabolism of phenols and phytoestrogens. All those selected single nucleotide polymorphisms (SNPs), located in exons or UTRs, have been reported minor allele frequencies (MAF) of >0.05 in the Han Chinese population. In the case of multiple SNPs which were in linkage disequilibrium, only one was selected. Finally, we selected 23 potential functional polymorphisms in metabolic enzymes: *CYP1A1* rs1048943 and rs4646422; *CYP1B1* rs2855658 and rs9341266; *CYP2B6* rs3760657, rs2054675, rs707265, and rs1042389; *CYP2C8* rs1058932; *CYP2C9* rs4918758; *CYP2C19* rs3814637, rs4986894, and rs3758581; *CYP2E1* rs2031920; *CYP2S1* rs3810171 and rs338583; *NAT1* rs7845127 and rs10888150; *NAT2* rs1799930, rs1799931 and rs4646246; *SULT1E1* rs4149525 and rs3736599. In addition, we also included 2 null polymorphisms in *GSTT1* and *GSTM1* here. SNPs were genotyped using TaqMan SNP Genotyping Assays (Biosteed, Nanjing, China) or GenomeLab SNPstream high throughput 12-plex genotyping platform (Beckman Coulter, Fullerton, CA)(Qin et al., 2014), and null polymorphisms were identified using PCR reactions(Wu et al., 2013). For quality control, 10% of the samples were randomly genotyped again, and the repeatability was 100%.

2.4. Metabolomic analysis

We randomly selected 31 urine samples to perform metabolomic analysis. The mixture of 200 μL urine and 400 μL methanol was collected in a 1.5 mL Eppendorf tube. After centrifugation at 16,000 *g* for 15 min, supernatant was obtained and then dried in a centrifugal concentrator. The residue was reconstituted in 400 μL ddH_2O. Metabolomic analysis was based on an accurate and sensitive method previously reported by Blasco et al. (2013). Briefly, LC-HRMS analysis was performed on an UPLC Ultimate 3000 system (Dionex), coupled with a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The system was controlled by Xcalibur 2.3 (Thermo Fisher Scientific). During the full-scan acquisition which ranged from 70 to 1050 *m/z*, the instrument operated at a 70,000 resolution. A multistep gradient had mobile phase A of 0.1% formic acid in ultra-pure water and mobile phase B consisting of acetonitrile acidified with 0.1% formic acid; the gradient operated at a flow rate of 0.4 mL/min over a run time of 15 min. All samples were analyzed randomly in case of the bias from the injection order. And identification was based on the accurate mass and the retention time of metabolites compared with those of standards.

2.5. Statistical analyses

There was a total of 1039 subjects available in this study. All statistical analyses were conducted using R 3.1.2 (Foundation for Statistical Computing, Vienna, Austria). A *P-value* of ≤0.05 was considered as the threshold for statistical significance. In statistical hypothesis testing, a type I error is the incorrect rejection of a true null hypothesis, and it is more likely to occur when considering a set of inferences simultaneously. To prevent this error, we used a statistical technique called the Bonferroni correction to get a higher significance threshold (*P-value* ≤ 1.67×10^{-4}) for individual comparisons, which compensated for the number of inferences being made. In descriptive analyses, we explored the mean age, BMI and constituent ratios of smoking status, alcohol consumption and tea consumption in all subjects. Values of urinary EDC concentrations below LODs were imputed as LOD/2. Additionally, the creatinine correction was used to balance variations of urinary dilution in samples, so exposure value in this study was presented as concentrations per gram of creatinine. To examine potential interactions