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Preliminary examination of polymorphisms of *GSTM1*, *GSTT1*, and *GSTZ1* in relation to semen quality☆

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ABSTRACT

Background: Environmental, lifestyle, and occupational exposures on semen quality have been investigated in epidemiological studies with inconsistent results. Genetic factors involved in toxicant activation and detoxification have been examined in relation to the risk of outcomes such as cancer, cardiovascular, and neurologic disorders. However, the effect of common genetic variants in the metabolism of toxicants on semen quality parameters has rarely been evaluated. In this analysis, we evaluated functional SNPs of three genes of the glutathione-S-transferase (GSTM1, GSTT1, GSTZ1) enzyme family.
Methods: Participants were 228 presumed fertile men recruited as part of a community-based study. Semen outcome data from this study included total sperm count and concentration, sperm morphology, and sperm DNA integrity and chromatin maturity. DNA was obtained from 162 men from a mouth-rinse sample and genotyped for the presence of *GSTT1-1* and *GSTM1-1* null genotypes and the *GSTZ1* SNPs at positions 94 (rs3177427) and 124 (rs3177429). We used multivariable linear regression to assess the relationship between each genotype and sperm outcomes.
Results: Overall, our results did not reveal a consistent pattern between *GSTM1* and *GSTZ* genotypes and increased occurrence of adverse sperm outcomes. However, the *GSTT1* non-null genotype yielded the coefficients with the largest magnitude for sperm count and sperm concentration ($\beta = -0.528$, 95% CI -1.238 to 0.199 and $\beta = -0.353$, 95% CI -0.708 to 0.001, respectively), suggesting that it might be adverse.
Conclusions: These results indicate that common polymorphisms in GST genes do not negatively impact sperm parameters in healthy men with good semen quality. Contrary to expectations, the *GSTT1* non-null genotype was associated with reduced sperm concentration and count in semen. Further study with a larger study size and inclusion of gene-exposure interactions is warranted.

1. Introduction

The potential impacts of environmental, lifestyle, and occupational exposures on semen quality have been examined in various epidemiological studies [1–9]. Some studies have been limited to routine semen outcomes such as sperm counts, motility, and morphology, whereas more recent studies have also included measures of sperm DNA, and chromosome or chromatin integrity [10–14]. Although mechanistic pathways are rarely examined in these studies, it is suspected that the exposure effects may be mediated through inherited genetic factors involved in toxicant activation and detoxification.

An important enzyme family involved in the detoxification of reactive intermediates is glutathione-S-transferase (GST). These enzymes are involved in the conjugation of reactive intermediates with glutathione, facilitating excretion, and are generally protective. However, GST theta has also been shown to activate some substrates to reactive intermediates that are mutagenic. For

Abbreviations: CMA, chromomycin A3; DCA, dichloroacetic acid; DDE, organochlorine; DDT, organochlorine; GA, heterozygous variant carriers; GST, glutathione-S-transferase; GSTM1, coding for GST mu1; GSTT1, coding for GST theta1; GSTZ, coding for GST zeta; GSTZ1, protein; GSTZ1A, isoform protein; HMS, Healthy Men Study; ROS, reactive oxygen species; SCSA, sperm chromatin structure assay.

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example GST theta (GSTT1-1) activates the brominated trihalomethanes, which are disinfection byproducts present in drinking water [15].

Several GST enzymes are encoded by genes with known functional polymorphisms. For example, deletions in *GSTM1* (coding for GST mu1) and *GSTT1* (coding for GST theta1) are relatively common in human populations [16]. Homozygous deletions *GSTM1*0* and *GSTT1*0* result in a lack of enzyme activity [17,18]. These polymorphisms have been associated with an increased risk of cancer, heart disease, and adverse reproductive outcomes [19–21]. In addition, *GSTM1*0* has been examined in relation to semen quality and infertility in a small number of studies [22–24]. GSTZ (coding for GST zeta) catalyzes the glutathione-dependent biotransformation of α-haloacetic acids, including dichloroacetic acid (DCA), a disinfection byproduct, to glyoxylic acid [25]. Promoter and non-synonymous exonic SNPs of the human *GSTZ* have been identified [26–28], resulting in five known isoforms of the GSTZ1 protein. These protein isoforms are a result of four non-synonomous SNPs (located at the nucleotides positions 23, 94, 124, and 245 corresponding to amino acids 8, 32, 42, and 82). Isoform GSTZ1A contains a Lys rather than a Glu at amino acid 32 and an Arg rather than a Gly at amino acid 42. This isoform has been shown to have increased activity for certain alpha-haloacid substrates, due primarily to an increase in the enzyme's resistance to inactivation by dichloroacetate (DCA) [26,27].

We examined the relationship between these common polymorphisms of *GSTM1*, *GSTT1*, and *GSTZ1* and semen quality, including sperm count, sperm concentration, sperm morphology, and sperm chromatin integrity, among a cohort of presumed fertile men.

2. Materials and methods

2.1. Study population

Genotype data were obtained on a subgroup of men who participated in the "Healthy Men Study" (HMS), a study of drinking water disinfection byproducts and semen quality. As has been described previously [14,29], HMS identified male partners of women who participated in a prospective study of drinking water disinfection byproducts and spontaneous abortion and other pregnancy outcomes, the "Right From the Start" study [30,31], conducted in three sites (Raleigh, NC; Memphis, TN; and Galveston, TX). A total of 228 men were included in the primary HMS analysis [14].

2.2. Semen collection and processing

The semen collection and analyses methods have been described in detail elsewhere [14,29]. Briefly, participants were asked to provide a single semen sample using a special kit designed to allow the man to collect a semen specimen in the privacy of his own home and at a time convenient to him [32]. All samples were packaged with cold packs (necessary to maintain sample stability for the sperm chromatin structure assay, SCSA) and shipped by overnight courier to a single laboratory at the U.S. EPA. Immediately upon receipt, semen volume was measured, and aliquots were removed for determination of sperm concentration by IVOS-IDENT (Hamilton Thorne Research, Beverly, MA.) [33], from which total sperm count was calculated. Additional aliquots were taken to prepare smears that were air-dried and stored for later analyses of sperm morphology [34]. Sperm motility, which declines over time and, therefore, is not a reliable measure for shipped semen, was not included in the statistical analysis. Additional aliquots (0.1 ml) were frozen and stored at −70 °C for later analysis of chromatin integrity by SCSA [35], and for chromatin immaturity (protamine deficiency indicated by chromomycin A3 (CMA) staining) [36].

2.3. Sperm outcome measures

In this analysis we focused on the following sperm outcomes: sperm count (million/sample) and sperm concentration (million/ml semen), sperm morphology (% normal sperm) including its components (percent of sperm cells with abnormal head, percent of sperm cells with abnormal midsection, percent of sperm cells with abnormal tails, and percent of sperm cells with abnormal cytoplasmic drop), percent sperm with DNA fragmentation, indicative of DNA damage, according to SCSA (%DFI), and % sperm with immature chromatin according to CMA staining.

2.4. Mouth-rinse collection

The present study of genetic polymorphisms was added to the primary HMS project. We re-contacted and recruited approximately 230 male participants in all three study sites who had successfully completed all of the parent HMS study activities (i.e., provided a signed consent form, completed telephone interview, and provided a donation of semen specimen according to study protocol). Men who had declined originally to participate in HMS, dropped out, or failed to complete all study activities were not re-contacted. Once an HMS participant agreed to participate in the polymorphism study, we scheduled the mailing of a mouth-rinse kit for buccal cell collection. At the end of recruitment and follow-up period, 227 recruitment letters were mailed, 188 agreed to participate, and 162 returned their specimen collection kits (response frequency: 162/188 = 86.2%). DNA was isolated from buccal cells by a standard high salt extraction method using Puregene chemistries (Qiagen, Valencia, CA).

2.5. Sample processing and genotyping

A multiplex PCR was performed to analyze for the presence of *GSTT1-1* and *GSTM1-1* [37], and an additional method [38] was also used to confirm the *GSTT1-1* genotype. Genotyping of the *GSTZ1* SNPs at positions 94 (rs3177427) and 124 (rs3177429) was performed on an Applied Biosystems (ABI) 7300 machine using ABI Pre-Designed/Validated TaqMan MGB probes and primers and TaqMan Universal Master Mix (no UNG) and analyzed using ABI software version 2.2. All samples were run in duplicate, and concordance between duplicates was 100%. Out of 162 samples, 2 could not be genotyped for rs3177427.

2.6. Statistical analyses

Several of the outcome variables were transformed to better approximate the normality assumption of the linear model. Specifically, a natural log transformation was applied to the sperm count and concentration variables, and an arc sine transformation was applied to the percent normal sperm cells, percent of sperm cells with abnormal head, percent of sperm cells with abnormal midsection, percent of sperm cells with abnormal tails, and percent of sperm cells with abnormal cytoplasmic drop. Linear regression was used to assess the association between each genotype variable and each outcome, adjusted for potential confounders (race/ethnicity, age, and study site). Abstinence interval was examined as a potential confounder but was not significant in the model. Although abstinence is related to the semen quality measures, it is not associated with genotype. Thus, it would not be expected to confound the relationship of interest. We also examined history of cigarette smoking (ever/never, amount, and ever/never is the 90 days before semen sample collection), but its adjustment did not materially alter the regression coefficients. For interpretability, each of the outcome variables was standardized (after statistical transformation, if applied) such that the standard deviation and the variance were equal to one. Thus each beta coefficient provides an estimate of effect in terms of a change in standard deviations of the transformed response variable. We used SAS version 9.1 software (Cary, NC) to perform all analyses.

3. Results

The subjects included in the genetic analyses were primarily from Memphis and Raleigh sites (69 and 67, respectively), with only 26 (16%) coming from the Galveston site (Table 1). The majority of the men was white, non-Hispanic, between 25 and 34 years old, and had a college degree. Over 80% of the subjects had household incomes of $40,000 or more. About one-third of the subjects reported smoking cigarettes. Subjects who agreed to participate in the genetic analyses were more likely to be white, non-Hispanic, better educated, and to smoke cigarettes when compared to all of the men eligible for the original HMS study.

Table 2 presents the self-reported racial and ethnic characteristics of the final participants stratified by genotype. None of the participants with the *GSTM1*0* null genotype were Black or Hispanic. Similarly, the *GSTT1*0* null genotype was found only among non-Hispanic participants. None of the heterozygous variant carriers (GA) of the *GSTZ1* SNP 124 were Hispanic. Furthermore, the only carriers of the homozygous variant (AA) of the *GSTZ1* SNP 94 and SNP 124 were white.

Although this study was motivated by reported impacts of GST polymorphisms and metabolism of disinfectant byproducts (mainly brominated trihalomethanes and haloacetic acids), the number of participants was not sufficient to examine interactions with disinfectant byproduct exposures. Therefore, we examined