Interactions between Urinary 4-tert-Octylphenol Levels and Metabolism Enzyme Gene Variants on Idiopathic Male Infertility

Yufeng Qin1,2,*, Minjian Chen1,2,*, Wei Wu1,2,*, Bin Xu1,2, Rong Tang1,2, Xiaojiao Chen1,2, Guizhen Du1,2, Chuncheng Lu1,2, John D. Meeker3, Zuomin Zhou1, Yankai Xia1,2, Xinru Wang1,2*

1 State Key Laboratory of Reproductive Medicine, Institute of Toxicology, Nanjing Medical University, Nanjing, China, 2 Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, China, 3 Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, United States of America

Abstract

Octylphenol (OP) and Trichlorophenol (TCP) act as endocrine disruptors and have effects on male reproductive function. We studied the interactions between 4-tert-Octylphenol (4-t-OP), 4-n-Octylphenol (4-n-OP), 2,3,4-Trichlorophenol (2,3,4-TCP), 2,4,5-Trichlorophenol (2,4,5-TCP) urinary exposure levels and polymorphisms in selected xenobiotic metabolism enzyme genes among 589 idiopathic male infertile patients and 396 controls in a Han-Chinese population. Ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to measure alkylphenols and chlorophenols in urine. Polymorphisms were genotyped using the SNPstream platform and the Taqman method. Among four phenols that were detected, we found that only exposure to 4-t-OP increased the risk of male infertility (P_trend = 1.70 × 10^{-7}). The strongest interaction was between 4-t-OP and rs4918758 in CYP2C9 (P_inter = 6.05 × 10^{-7}). It presented a significant monotonic increase in risk estimates for male infertility with increasing 4-t-OP exposure levels among men with TC/CC genotype (low level compared with non-exposed, odds ratio (OR) = 2.26, 95% confidence intervals (CI) = 1.06, 4.83; high level compared with non-exposed, OR = 9.22, 95% CI = 2.78, 30.59), but no associations observed among men with TT genotype. We also found interactions between 4-t-OP and rs4986894 in CYP2C19, and between rs1048943 in CYP1A1, on male infertile risk (P_inter = 8.09 × 10^{-7}, P_inter = 3.73 × 10^{-4}, respectively). We observed notable interactions between 4-t-OP exposure and metabolism enzyme gene polymorphisms on idiopathic infertility in Han-Chinese men.

Citation: Qin Y, Chen M, Wu W, Xu B, Tang R, et al. (2013) Interactions between Urinary 4-tert-Octylphenol Levels and Metabolism Enzyme Gene Variants on Idiopathic Male Infertility. PLoS ONE 8(3): e59398. doi:10.1371/journal.pone.0059398

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: yankaixia@njmu.edu.cn (YX); xrwang@njmu.edu.cn (XW)

These authors contributed equally to this work.
activity [20]. Genetic variants can also modify the catalytic activity of enzymes [21,22]. Thus, susceptibility to the effects of phenols exposure may vary between individuals, possibly due to polymorphisms in metabolizing enzymes. To our knowledge, no studies have investigated the potential interaction between OPs and TCPs exposure and metabolism enzyme genes variants in relation to male infertility in Han-Chinese population. In this study, we assessed relationships between urinary concentrations of several OPs and TCPs and male infertility for the first time. We also studied the potential interaction of OPs, TCPs and variants in metabolism enzyme genes on male infertility.

Materials and Methods

Study Population and Sample Collection

The protocol and consent form were approved by the Institutional Review Board of Nanjing Medical University prior to the study. Fertile controls and male infertility patients were consecutively recruited from the Affiliated Hospitals of Nanjing Medical University from 2005 to 2008. The patients had been unable to conceive for at least 12 months (without diagnosed infertile wives) and had undergone a complete historical and physical examination. The control subjects were fertile men from the early pregnancy registry of the same hospitals who were in the third month following a successful pregnancy. They were healthy men with normal reproductive function and confirmed having healthy babies 6–8 months later [23]. All activities involving human subjects were done under full compliance with government policies and the Helsinki Declaration. Consecutive eligible men were recruited to participate, 1317 in total were asked. Of those approached, 89.2% consented (1175 participants; 707 cases and 468 controls). After the study procedures were explained and all questions were answered, subjects signed informed consent forms. A completed physical examination including height and weight was performed, and a questionnaire was used to collect information including personal background, lifestyle factors, occupational and environmental exposures, genetic risk factors, sexual and reproduction status, medical history and physical activity. Men with abnormal sexual and ejaculatory functions, immune infertility, semen non-liquefaction, medical history of risk factors for infertility (e.g., varicocele, postvasectomy or orchidopexy), and receiving treatment for infertility (e.g., hormonal treatments) were excluded from the study (54 of 707 subjects). Men with other known causes related to male infertility, such as genetic disease, infection, or occupational exposure to agents suspected to be associated with male reproduction, were also excluded (34 of 653 subjects). Furthermore, to avoid azoospermia or severe oligozoospermia caused by Y chromosome microdeletions, we excluded subjects with Y chromosome microdeletions of Azoospermia Factor (AZF) region (11 of 619 subjects, microdeletion rate was 1.78%). Those subjects who declined to leave both blood samples and urine samples were excluded in our study. We also excluded the samples that did not pass quality control checks. In total 396 fertile controls and 589 infertile patients were included in this study and claimed that their life styles and environments had not changed for several months leading up to sample collection. After completing a questionnaire including detailed information, such as age, cigarette smoking, and alcohol consumption, each subject donated 5 ml of blood which was used for genomic DNA extraction and a urine sample for measuring the concentrations of 4-t-OP, 4-n-OP, 2,3,4-TCP and 2,4,5-TCP.

Exposure Assessment

We measured total urinary concentrations of 4-t-OP, 4-n-OP, 2,3,4-TCP and 2,4,5-TCP using ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Briefly, urine samples were incubated in 1 M ammonium acetate buffer solution (pH = 5.0) for hydrolysis with β-glucuronidase/ sulfatase (20000 units/mL) overnight. After hydrolysis, the phenols were extracted and preconcentrated with solid phase extraction (500 mg/3 mL, Supelclean, USA), and determined with UPLC (Acuity UPLCTM 6 BEH C18 column, 1.7 μm, 2.1×100 mm) electrospray ionization (negative ion mode)-MS/MS. The limits of detection (LOD) were 0.28 μg/L (2,3,4-TTCP), 0.15 μg/L (2,4,5-TCP), 0.34 μg/L (4-t-OP) and 0.02 μg/L (4-n-OP). Creatinine concentrations were analyzed using an automated chemistry analyzer (7020 Hitachi, Tokyo, Japan).

SNP Selection and Genotype Analyses

Through information gained from PubMed and Hapmap, we identified twenty potential functional polymorphisms in metabolism enzymes: CYP1A1 rs1049843, CYP2B6 rs7360657, rs2054675, rs707265 and rs1042389, CYP2C8 rs1058932, CYP2C9 rs4918758, CYP2C19 rs3814637, rs4986894 and rs11568732, CYP2S1 rs3810171 and rs335838, NAT1 rs7845127 and rs10888150, NAT2 rs1799930, rs1799931, rs4646246 and rs4646243, SULT1E1 rs4149525 and rs3736599 (Table 1). All selected single nucleotide polymorphism (SNPs) have reported minor allele frequencies (MAF) of >0.05 in Han-Chinese population and are located in potential functional areas. In the case of multiple SNPs in the same haplotype block (linkage coefficient r^2>0.8), only one was selected. Eight SNPs (rs2054675, rs707265, rs1042389, rs4986894, rs11568732, rs335838, rs10888150, rs1799931) were genotyped by using TaqMan SNP Genotyping Assays (Bioseed, Nanjing, China) and the other twelve SNPs (rs1048943, rs3760657, rs1058932, rs4918758, rs3814637, rs3810171, rs7845127, rs1799930, rs4646246, rs4646243, rs4149525 and rs3736599) were genotyped by GenomeLab SNPStream high throughput 12-plex genotyping Platform (Beckman Coulter, Fullerton, CA) following the manufacturer’s instructions [24]. For quality control, 10% of the samples were randomly genotyped again, and the repeatability was 100%.

Table 1. Characteristics of the cases and controls.

| Variable | Controls (n = 396) | Cases (n = 589) |
|----------|------------------|----------------|
| Age (years, mean ± SD) | 29.75±3.43 | 38.41±4.61* |
| Smoking status | | |
| Ever | 189 (52.1) | 306 (52.4) |
| Never | 174 (47.9) | 278 (47.6) |
| Drinking | | |
| Yes | 184 (51.0) | 299 (51.1) |
| No | 177 (49.0) | 286 (48.9) |
| BMI | 23.73±3.33 | 23.26±3.19* |

^p<0.05 for T test or two-side X^2 test for selected characteristics distributions between control and case groups.

doi:10.1371/journal.pone.0059398.t001

Statistical Analyses

Statistical analyses were carried out using Stata 10.0 statistical software package [Stata Corp, LP]. Pearson’s chi-squared test was used to test the differences of categorical variables such as drinking and smoking status between cases and controls. Student’s t-test was used to test for differences in continuous variables such as age and body mass index.
body mass index (BMI) between groups. For SNP main effects analysis, we used additive genetic models. For exposure main effects analysis and interaction analysis, we categorized exposure variables ordinally (none/low/high). Men with urinary exposure level below the LOD were the reference group (non-exposed) and those with urinary exposure levels above the LOD were categorized into two groups using the urinary concentration median among samples with detectable levels as the cut-point separating “low” and “high”.

Associations between exposure and the risk of male infertility were evaluated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analyses with adjustment for age, BMI and urinary creatinine. The joint effects of exposure were evaluated by using multivariate logistic regression to calculate the odds of infertility associated with detection of multiple chemicals in urine, adjusted for age and BMI, with the

Table 2. Distribution of 4-t-OP, 4-n-OP, 2,3,4-TCP, and 2,4,5-TCP concentrations in urine (µg/L) in Han-Chinese males (n = 985).

| Analytes   | Geometric mean(95%CI) | percentile 50th | 75th | 90th | 95th | 99th |
|------------|-----------------------|-----------------|------|------|------|------|
| 4-t-OP     | 0.60 (0.56–0.64)      | < LOD           |     |      |      |      |
| 4-n-OP     | 0.05 (0.05–0.06)      | < LOD           | 0.03 |      | 0.08 | 0.11 |
| 2,3,4-TCP  | 0.51 (0.48–0.59)      | < LOD           |      |      |      | 0.43 |
| 2,4,5-TCP  | 0.32 (0.29–0.35)      | < LOD           | 0.24 |      | 0.37 | 0.85 |

LOD for 4-t-OP, 4-n-OP, 2, 3, 4-TCP and 2, 4, 5-TCP were 0.34 µg/L, 0.02 µg/L, 0.28 µg/L, and 0.15 µg/L, respectively.

doi:10.1371/journal.pone.0059398.g002
non-exposed group as a reference. We used QVALUE software to calculate false discovery rate (FDR)-adjusted P value [25].

Potential gene-environment interactions were tested by comparing the changes in deviance (∆ log likelihood) between models of main effects with or without the interaction term. Stepwise regression was used to avoid interference and identify the most important interactions. We used the homozygous wild-type, non-exposed men as the reference category. The ORs and 95% CIs for the remaining genotype-exposure categories were estimated. An alpha of 0.05 was considered the threshold of significance.

Results

Characteristics of the Study Population

The study contained 589 infertile male patients and 396 control men proven fertility. No significant differences were identified between case and control groups with regard to smoking and drinking status. However, there were significant differences in age and BMI between cases and controls (Table 1). The mean (± SD) age and BMI were 29.75±3.43 years and 23.73±3.33 in control group, respectively. The mean (± SD) age and BMI in case group were 28.41±4.61 years and 23.26±3.19.

Associations between Urinary Exposure Levels and Male Infertility

The structure of 4-t-OP, 4-n-OP, 2,3,4-TCP, 2,4,5-TCP are presented in Figure 1, and distributions of their urinary concentrations in the 985 participants are presented in Table 2. Geometric means of 4-t-OP, 4-n-OP, 2,3,4-TCP and 2,4,5-TCP were 0.60 μg/L, 0.05 μg/L, 0.53 μg/L and 0.32 μg/L, respectively. There were no associations between 4-n-OP, 2,3,4-TCP, or 2,4,5-TCP and male infertility. High exposure to 4-t-OP was significantly associated with male infertility (P\text{trend} = 1.70×10^{-7}, OR = 4.05, 95% CI = 2.08–7.87) (Figure 2, Table S1). Considering the possibility of risk from being exposed to multiple chemicals, we analyzed the joint effect of these four phenols on male infertility. We found that men exposed to more than one chemical had significantly elevated odds of infertility (P\text{trend} = 0.008) (Table 3).

Associations between Polymorphisms and Male Infertility

A total of 20 SNPs in metabolism enzyme genes were investigated in our study. Results for SNP main effects in relation to male infertility are presented in Table 4. However, no significant associations with male infertility were retained after FDR adjustment.

Interaction between Exposure Level and Polymorphisms on Male Infertility

We tested for interactions between 4-t-OP and metabolism enzyme gene polymorphisms. About twenty interactions that were tested met the FDR-adjusted P value <0.05 criterion in total (Table S2). We then used stepwise regression to avoid interferences and to identify the most important interactions. In the end, interactions between 4-t-OP and rs4198758, rs4918758 and rs1048943 were retained (Table 5). The strongest interaction was between 4-t-OP and rs4918758 in CYP2C9 (P\text{trend} = 6.05×10^{-7}). Odds for infertility were significantly increased with increasing 4-t-OP exposure among men with TC/CC

Table 3. Joint effects of urinary 4-t-OP, 4-n-OP, 2,3,4-TCP and 2,4,5-TCP Levels and male infertility (n = 985).

| No. of exposed chemicals | Ca/Co | OR(95%CI)a | Pb |
|-------------------------|-------|------------|----|
| Reference group         | 229/179 | 1.00(reference) |   |
| 1                       | 243/163  | 1.26(0.94–1.69)  | 0.121 |
| ≥2                      | 117/54   | 1.77(1.19–2.62)  | 0.010 |

Ca: Cases; Co: Controls.

P: False Discovery Rate-corrected P value.

aAdjusted for age, BMI and creatinine.

Interaction between Exposure Level and Polymorphisms on Male Infertility

We tested for interactions between 4-t-OP and metabolism enzyme gene polymorphisms. About twenty interactions that were tested met the FDR-adjusted P value <0.05 criterion in total (Table S2). We then used stepwise regression to avoid interferences and to identify the most important interactions. In the end, interactions between 4-t-OP and rs4198758, rs4918758 and rs1048943 were retained (Table 5). The strongest interaction was between 4-t-OP and rs4918758 in CYP2C9 (P\text{trend} = 6.05×10^{-7}). Odds for infertility were significantly increased with increasing 4-t-OP exposure among men with TC/CC

Table 4. Associations between metabolism enzyme gene SNPs and male infertility.

| Gene     | SNP     | Position | Nucleotide change | MAFa | OR(95%CI)bPc | Pd |
|----------|---------|----------|------------------|------|---------------|----|
| CYP1A1   | rs1048943 | rsSNP    | A>G              | 0.256 | 1.08(0.52–2.25) | 0.938 |
| CYP2B6   | rs3760657 | 5F       | A>G              | 0.146 | 0.76(0.38–1.52) | 0.938 |
| CYP2B6   | rs2054675 | 5F       | T>C              | 0.185 | 1.73(0.76–3.98) | 0.776 |
| CYP2B6   | rs707265  | 5F       | G>A              | 0.341 | 0.96(0.63–1.45) | 0.938 |
| CYP2C8   | rs1042389 | 3’UTR    | T>C              | 0.314 | 0.93(0.58–1.51) | 0.938 |
| CYP2C9   | rs4918758 | 5F       | T>C              | 0.337 | 0.87(0.57–1.31) | 0.938 |
| CYP2C9   | rs3814637 | 5F       | C>T              | 0.116 | 1.06(0.29–3.92) | 0.977 |
| CYP2C9   | rs4986894 | 5F       | T>C              | 0.244 | 0.91(0.56–1.47) | 0.938 |
| CYP2C9   | rs11568732| 5F       | T>G              | 0.093 | 2.46(0.66–9.19) | 0.776 |
| CYP2S1   | rs3810171 | 5F       | C>T              | 0.100 | 7.51(0.96–58.89) | 0.730 |
| CYP2S1   | rs338583  | 3’UTR    | T>C              | 0.195 | 1.00(0.46–2.19) | 0.992 |
| NAT1     | rs7845127 | 5F       | C>T              | 0.407 | 1.18(0.77–1.82) | 0.938 |
| NAT1     | rs10888150| 5F       | T>C              | 0.442 | 0.95(0.65–1.39) | 0.938 |
| NAT2     | rs1799930 | rsSNP    | G>A              | 0.167 | 1.17(0.56–2.42) | 0.938 |
| NAT2     | rs1799931 | rsSNP    | G>A              | 0.222 | 0.65(0.28–1.51) | 0.938 |
| NAT2     | rs4646246 | 5F       | G>A              | 0.488 | 1.44(0.97–2.15) | 0.730 |
| NAT2     | rs4646243 | 5F       | C>T              | 0.411 | 0.76(0.51–1.12) | 0.776 |
| SULT1E1  | rs4149525 | 5F       | A>G              | 0.267 | 0.95(0.58–1.55) | 0.938 |
| SULT1E1  | rs3736599 | 5F       | G>A              | 0.226 | 1.04(0.79–1.36) | 0.938 |

aMinimum allele frequency in the general Han Chinese population.

bAdditive genetic model was used.

cFalse Discovery Rate-corrected P value.

dAdjusted for age and BMI.

Interaction between Exposure Level and Polymorphisms on Male Infertility

We tested for interactions between 4-t-OP and metabolism enzyme gene polymorphisms. About twenty interactions that were tested met the FDR-adjusted P value <0.05 criterion in total (Table S2). We then used stepwise regression to avoid interferences and to identify the most important interactions. In the end, interactions between 4-t-OP and rs4198758, rs4918758 and rs1048943 were retained (Table 5). The strongest interaction was between 4-t-OP and rs4918758 in CYP2C9 (P\text{trend} = 6.05×10^{-7}). Odds for infertility were significantly increased with increasing 4-t-OP exposure among men with TC/CC
We observed a significant interaction compared with non-exposed [OR = 2.56, 95%CI = 0.91, 7.16, for high level compared with non-exposed, OR = 9.22, 95%CI = 2.78, 30.59; but no association among with TT genotype for low exposure level compared with non-exposed, OR = 4.32, 95%CI = 1.23, 15.18; for high level compared with non-exposed, OR = 3.44, 95%CI = 1.56, 7.57 in AA genotype group; for low level compared with non-exposed, OR = 5.47, 95%CI = 1.59, 18.92 in AG/GG genotype].

### Discussion

Our study is the first study to evaluate the interaction between phenols exposure and metabolism enzyme polymorphisms in male infertility risk. In multivariable analyses for main effects, high exposure to 4-t-OP was associated with significant elevations in the odds of male infertility, while there were no significant associations between metabolism enzyme polymorphisms and male infertility. Three interactions were observed between4-t-OP and metabolism gene polymorphisms following adjustment.

OPs are widely used in industrial manufacturing and can be detected in the environment. Exposure of male rats to 4-t-OP caused increased sperm abnormalities and decreased sperm number [8]. 4-n-OP has also demonstrated estrogenic and antiandrogenic effects in vitro [7]. Due to the different structure of the branched alkyl chains, there are conflicting opinions about the toxicity of the 4-t-OP and 4-n-OP [26,27]. Some groups have reported that 4-t-OP is the most estrogenic of the 4-alkylphenols, and in vitro studies have demonstrated that it binds to the estrogen receptor and activates estrogen-responsive genes [28,29]. Sachiko Nomura used the rat liver to elucidate the metabolism of 4-t-OP and 4-n-OP depended on the shape of the alkyl chains. 4-t-OP is circulated between the liver and intestine, suggesting that a continuous exposure of the target organs to the chemical occurs [30]. This may be consistent with our results, where exposure to 4-t-OP, but not 4-n-OP, was found to be associated with increased odds of male infertility.

It is known that common diseases have complex etiologies related not only to genetic factors but also environmental factors. Recently, there has been increased interest in gene-environment interactions, which may affect infertility pathophysiology [31,32]. Thus, we also studied the interactions between phenols exposure and the genetic variants in metabolism enzymes on male infertility.

![Table 5](image)

Table 5. Interactions between urinary 4-t-OP, 4-n-OP, 2,3,4-TCP and 2,4,5-TCP levels and polymorphisms on male infertility.

| Analytes | SNPs | Genotype | 4-t-OP exposure level | OR(95%CI)* | OR(95%CI)* |
|----------|------|----------|-----------------------|------------|------------|
|          |      |          | None | Low | High | None | Low | High |
| 4-t-OP   |      |          | Ca/Co | Ca/Co | Ca/Co | Ca/Co | Ca/Co | Ca/Co |
| CYP2C9   | rs4918758 | TT | 163/115 | 19/7 | 2.56(0.91–7.16) | 18/9 | 1.62(0.67–3.96) | 6.05 x 10^-7 |
|          |      | TC+CC | 307/242 | 37/10 | 2.26(1.06–4.83) | 43/4 | 9.22(2.78–30.59) |                |
| CYP2C19  | rs4986894 | TT | 230/153 | 26/9 | 2.06(0.86–4.96) | 28/9 | 1.99(0.90–4.42) | 8.09 x 10^-7 |
|          |      | TC+CC | 222/191 | 25/7 | 2.11(0.87–5.13) | 30/2 | 17.35(2.33–129.04) |                |
| CYP1A1   | rs1048943 | AA | 291/228 | 35/13 | 2.00(1.00–4.00) | 39/9 | 3.44(1.56–7.57) | 3.73 x 10^-4 |
|          |      | AG+GG | 179/132 | 19/4 | 4.32(1.23–15.18) | 21/4 | 5.47(1.59–18.92) |                |

None: non-exposed; Low: low level exposure; High: high level exposure; Ca: Cases; Co: Controls.

*Adjusted for age, BMI and creatinine.

False Discovery Rate-corrected P value.

P = 0.05.

P_inter for the stepwise regression

P_inter = 8.09

P_inter = 6.05 x 10^-7

P_inter = 3.73 x 10^-4

Niwa and colleagues reported that phenols exposure could inhibit the transcriptional activity of CYP2C9, which is a key member of CYP2C enzyme family, is responsible for the metabolism of drugs; genetic variants were reported to modify the enzyme activity, and subsequently cause toxicity [33–35]. Men carrying the rs4918758 minor allele may also have genetic susceptibility to male infertility risk when coupled with 4-t-OP exposure. We also found rs4986894 in CYP2C9 combined with 4-t-OP exposure increased the odds of male infertility (P_inter = 6.05 x 10^-7). It had a significant monotonic increase in male infertile risk with increasing 4-t-OP exposure in TC/CC genotype group, and no significant association in the TT genotype group. CYP2C9, which is also a member of CYP2C enzyme family, can metabolize drugs. Rs4986894 is located in the promoter of CYP2C9, and may modulate the transcriptional activity of CYP2C9. For rs1048943 in CYP1A1, exposure to 4-t-OP in both genotype groups was associated with significantly increased odds of male infertility compared with the non-exposure group. CYP1A1, coding the enzyme aryl hydrocarbon hydroxylase, is believed to participate in the metabolism process of certain chemicals, which can induce bulky DNA adducts in human sperm, diminished semen quality, and may result in male infertility [36,37]. Genetic variants in CYP1A1 can influence the activity of this enzyme [38]. The rs1048943 polymorphism, an A to G substitution causing an Ile to Val amino acid exchange at codon 462, showed a significant higher catalytic activity for estrogens than the wild-type enzyme [38].

4-t-OP, SNPs and Male Infertility
References

1. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL (2008) Exposure of the U.S. population to bisphenol A and 4-tert-octylphenol: 2003-2004. Environ Health Perspect 116: 39–44.
2. David A, Fenet H, Gomes E (2009) Alkylphenols in marine environments: distribution monitoring strategies and detection considerations. Mar Pollut Bull 58: 953–960.
3. Ying GG (2006) Fate, behavior and effects of surfactants and their degradation products in the environment. Environ Int 32: 417–431.
4. Colborn T, vom Saal FS, Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101: 378–384.
5. Sumpter JP (1998) Xenobiotic disrupters-environmental impacts. Toxicol Lett 102–103: 337–342.

Conclusion

We found exposure to 4-t-OP could increase the male infertile risk and we also observed notable interactions between 4-t-OP exposure and gene polymorphisms on risk of male infertility.

Supporting Information

Table S1 Associations between urinary 4-t-OP, 4-n-OP, 2,3,4-TCP, and 2,4,5-TCP levels and Male Infertility.

Table S2 Interactions between urinary 4-t-OP, 4-n-OP, 2, 3, 4-TCP, 2, 4, 5-TCP levels and polymorphisms on Male Infertility.

Author Contributions

Conceived and designed the experiments: YX XW. Performed the experiments: YQ MC. Analyzed the data: YQ WW BX. Contributed reagents/materials/analysis tools: RT XC GD CL ZZ. Wrote the paper: YQ JDM YX XW.
36. Shimada T, Fujii-Kuriyama Y (2004) Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci 95: 1–6.

37. Ji G, Gu A, Zhou Y, Shi X, Xia Y, et al. (2010) Interactions between exposure to environmental polycyclic aromatic hydrocarbons and DNA repair gene polymorphisms on bulky DNA adducts in human sperm. PLoS One 5 pii: e13145.

38. Kisselev P, Schunck WH, Roots I, Schwarz D (2005) Association of CYP1A1 polymorphisms with differential metabolic activation of 17beta-estradiol and estrone. Cancer Res 65: 2972–2978.

39. Akgul Y, Derk RC, Meighan T, Rao KM, Murono EP (2008) The methoxychlor metabolite, HPTE, directly inhibits the catalytic activity of cholesterol side-chain cleavage (P450sc) in cultured rat ovarian cells. Reprod Toxicol 25: 67–75.

40. Kim SK, Kim JH, Lee HJ, Yoon YD (2007) Octylphenol reduces the expressions of steroidogenic enzymes and testosterone production in mouse testis. Environ Toxicol 22: 449–458.