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Interactive Effect of Bisphenol A (BPA) Exposure with -22G/C Polymorphism in LOX Gene on the Risk of Osteosarcoma

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Abstract

Background: Osteosarcomas have many established risk factors, both genetic and environmental, but by themselves these explain only part of the total cancer incidence. Bisphenol A (BPA) is an environmental estrogen associated with risk of several kinds of tumour. The lysyl oxidase gene (LOX) may also contribute to risk of tumours including osteosarcomas. Here, we investigated possible interactions of BPA and a LOX polymorphism on the risk of osteosarcoma.

Method: The present hospital-based case-control study included 106 cancer patients and 112 controls from a Chinese population. Internal burden of BPA exposure was assessed using high-performance liquid chromatography–mass spectrometry (HPLC-MS) method. Genotypes were determined using PCR-RFLP methods.

Results: Compared with those in low BPA exposure group, subjects with BPA more than or equal to median value had significant increased risk of osteosarcoma among subjects who carried GC or CC genotypes. A significant interaction with BPA level and the -22G/C polymorphism was observed for osteosarcoma overall, osteosarcoma affecting knee and osteosarcoma affecting hip, as \( P_{\text{interaction}} = 0.036 \) for osteosarcoma overall; \( P_{\text{interaction}} = 0.024 \) for osteosarcoma affecting knee; and \( P_{\text{interaction}} = 0.017 \) for osteosarcoma affecting hip.

Conclusions: The results suggest that BPA exposure interacts with the -22G/C polymorphism of the LOX gene to increase the risk of osteosarcoma.

Keywords: Osteosarcoma - bisphenol A (BPA) - Lysyl oxidase gene (LOX) - gene polymorphism - interactive effect
polymorphism showed the most significantly increased risk of developing osteosarcoma, compared to wild-type genotype GG validated by recent study. It is possible that genetic variation in LOX of -22G>C polymorphism may modify the association between BPA exposure and osteosarcoma risk. As such, we conducted a hospital-based, case-control study in Chinese population to test this hypothesis.

Materials and Methods

Population
This hospital-based case-control study was carried out in Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The cases with osteosarcoma were recruited from January 2009 to July 2011. All patients of osteosarcoma were diagnosed by histopathological examination of biopsy or surgically resected specimen. The controls were selected from a pool of cancer-free subjects recruited from individual participating early cancer detection based on physical examination. There were no age, sex and stage restrictions; however, patients with previous cancer or metastasis from other origins were excluded. The selection criteria of control included no prior history of cancer, and controls were frequency matched to the cases by age (±2 years) and sex. This case–control study included 106 patients with osteosarcoma and 112 cancer-free controls. The mean age of the osteosarcoma patients was 16.13 ± 2.80 years (mean ± SD) and the control group was 15.89 ± 2.13 years. At recruitment, informed consent was obtained from each subject who was then interviewed for detailed information on demographic characteristics, alcohol consumption, family history and lifetime history of tobacco use. This study was approved by approved Ethics committee of Tongji Medical College, Huazhong University of Science and Technology.

Genotyping
Genomic DNA from all the subjects was extracted from the peripheral blood leukocytes according to a previous report and stored at -80 °C until use for genotyping. LOX genotype was analyzed by PCR-RFLP methods as described previously. The PCR primers for amplifying DNA fragment containing the -22G/C was: F 5'-GGGAACGCTCGTTGCTAAG-3'/R 5'-CTCCTATTATTCCCCAGGT-3'. A 10% random sample of cases and controls was repeated by different persons, and the results were found to be 100% concordant for all of the masked duplicate sets.

Exposure assessment
Urinary concentration of BPA was determined at School of Public Health, Tongji Medical College (Ministry of Education Key Laboratory of Environment and Health) using high-performance liquid chromatography–mass spectrometry (HPLC-MS) according to previous report. Briefly, 2.0 mL urine of each sample was used for the detection assay. The identification and quantification of BPA were based on retention time and peak area measured using a linear regression curve obtained from internal standard solutions. The detection limit of BPA was 0.5 ng/mL; for measurements below 0.5ng/mL, we used 0.35 ng/mL (70% of the detection limit) as the default. The valid urine BPA concentrations were expressed as micromoles per mole creatinine.

Statistical analysis
The frequency distributions of categorical variables were examined both in case and control groups by Pearson χ² test. The urine BPA exposure was divided into two groups according to the concentration examined. We defined persons as low exposure if their BPA level was less than median value and high exposure if their BPA level was more than or equal to median value. Unconditional logistic regression model was used to estimate the odds ratios (ORs) and 95% confidence intervals (Francis et al., 2003) for associations between BPA level, and risk of osteosarcoma and its subtypes in different genotype strata. Significance of gene-BPA exposure interaction was assessed by adding an interaction term in the logistic

### Table 1. Frequency Distribution of Selected Characteristics

| Variable          | Patients (n=106) | Controls (n=112) | P-value* |
|-------------------|-----------------|-----------------|----------|
| Sex               |                 |                 | 0.128    |
| Male              | 74   69.7        | 76   67.9        |          |
| Female            | 32   30.3        | 36   32.1        |          |
| Ethnicity         |                 |                 | 0.15     |
| Chinese Han       | 91   86          | 95   84.6        |          |
| Non-Chinese Han   | 15   14          | 17   15.4        |          |
| Age               |                 |                 | 0.216    |
| ≤ 15              | 37   34.6        | 39   35.1        |          |
| 15-18             | 54   50.8        | 55   49.9        |          |
| > 18              | 15   14.6        | 18   15          |          |
| Smoking status    |                 | <0.0001         |          |
| No                | 38   35.9        | 59   52.4        |          |
| Yes               | 68   64.1        | 53   47.6        |          |
| Site              |                 |                 |          |
| Knee              | 49   46.3        |                |          |
| Hip               | 38   35.9        |                |          |
| Others            | 19   17.8        |                |          |

### Table 2. Associations Between BPA and Risk of Osteosarcoma and Common Subtypes

| Variable          | Patients (n=106) | Controls (n=112) | P-value* |
|-------------------|-----------------|-----------------|----------|
| 1-OHP µmol/mol creatinine | <7.01 | ≥7.01 |          |
| Osteosarcoma overall |            |                 |          |
| Cases             | 43   63         |                |          |
| Control           | 55   57         |                |          |
| OR(95%CI)         | 1.41 (1.01-1.72)|                |          |
| P                 | 0.045           |                |          |
| Osteosarcoma affecting hip |       |                 |          |
| Case              | 14   22         |                |          |
| OR(95%CI)         | 2.00 (1.30-3.17)|                |          |
| P                 | 0.078           |                |          |
| Osteosarcoma affecting knee |        |                 |          |
| Case              | 21   36         |                |          |
| OR(95%CI)         | 1.66 (1.14-2.49)|                |          |
| P                 | 0.02            |                |          |
| Other sites       |                 |                 |          |
| Case              | 5    8          |                |          |
| OR(95%CI)         | 1.22 (0.71-1.41)|                |          |
| P                 | 0.082           |                |          |
Table 3. Associations Between LOX -22G/C Polymorphism, BPA, and Risk of Osteosarcoma

| LOX -22G/C polymorphisms | Osteosarcoma | Osteosarcoma affecting knee | Osteosarcoma affecting hip |
|--------------------------|--------------|-----------------------------|---------------------------|
|                          | BPA < 7.01   | BPA ≥ 7.01                  | BPA < 7.01                | BPA ≥ 7.01                |
| GG                       | Case Control | OR(95%CI)                   | Case Control | OR(95%CI) | Case Control | OR(95%CI) |
|                          | 33           | 40                          | 1                    | 42         | 35           | 1.37(1.00-7.15) |
|                          | 15           | 20                          | 1.38(1.01-7.21)       | 10          | 1            | 12.67(1.13-12.12) |
| GC or CC                 | 10           | 15                          | 1.48(1.06-7.37)       | 6           | 16           | 1.72(1.23-2.24) |
|                          | 4            |                              |                        | 4           | 1            | 10.24(1.45-3.36) |
| P_{interaction}          | 0.036        |                              |                        | 0.024       |               | 0.017      |

models. The Statistical Package for the Social Sciences (SPSS) software (version 13.0, SPSS, Inc., Chicago, Illinois) was used for the data analysis.

Results

Associations between BPA and risk of osteosarcoma and common subtypes

The study involved 106 patients with osteosarcoma and 123 non-neoplastic controls. The characteristics of the study population are provided in Table 1. The median value of urine BPA concentration in this study was 7.01 ng/ml. The association between BPA level and risk of osteosarcoma overall and osteosarcoma subtype are presented in Table 2. Compared with subjects in low exposure rank, those with BPA level more than 7.01 ng/ml had an increased risk of osteosarcoma overall (OR = 1.41; 95% CI, 1.01-1.72). After stratification by subtypes, an increased risk was observed for osteosarcoma affecting knee (OR = 1.66; 95% CI, 1.14-2.49) and osteosarcoma affecting hip (OR = 2.00; 95% CI, 1.30-3.17), but not for other parts (OR = 1.22; 95% CI, 0.71-1.41).

Associations between LOX -22G/C polymorphism, BPA, and risk of osteosarcoma

As shown in Table 3, a significantly increased risk of osteosarcoma was associated with urine BPA level among subjects who carried the variant of TC and CC polymorphism of LOX. Compared with the subjects whose BPA level were less than 7.01 ng/ml, subjects with higher BPA level had a more significantly increased risk of Osteosarcoma, if they carried TC or CC polymorphism of LOX. The P_{interaction} of BPA level and LOX genotype is statistical significant, as P_{interaction} = 0.036. A similar pattern was also observed for osteosarcoma affecting knee and osteosarcoma affecting hip. The interaction of -22G/C polymorphism and BPA was statistical significant, as P_{interaction} = 0.024 for osteosarcoma affecting knee and P_{interaction} = 0.017 for osteosarcoma affecting hip.

Discussion

Our study provided the first comprehensive analysis of interaction BPA exposure, genetic polymorphism in LOX gene, and risk of osteosarcoma and its subtypes. Significant interactions were observed for -22G/C polymorphism and BPA exposure for osteosarcoma risk overall, as well as osteosarcoma affecting knee and hip separately. Consistent with our hypothesis, the present study suggested that high BPA exposure was associated with an increased risk of osteosarcoma overall. BPA, as the most significant kind of synthetic xenoestrogen, can cause the disturbance of normal estrogen metabolism and involved in several kinds of tumors. In such a case, variants in normal estrogen signaling pathways may affect the interaction effects commonly generated by exposure to the BPA, and subsequently modify the association between BPA and risk of osteosarcoma. LOX, which is essential for the structural integrity and function of bone tissue (Eyre et al., 1988; Knott et al., 1995), is also participated in estrogen signal pathway related-osteosarcoma pathogenesis (Liu et al., 2012).

The study suggested that -22G/C polymorphism in LOX gene may have modified the relationship between BPA exposure and osteosarcoma risk. Especially, the result suggested the association of the C allele with increased risk of BPA related- osteosarcoma risk. Theoretically, if the LOX gene C allele has an increasing tumor triggering activity, individuals who carry this allele and are also exposed to BPA-induced hormone metabolism disorders will be at higher risk of osteosarcoma. This explanation was consistent with present study based on the analysis of interaction effect of BPA exposure and -22G/C polymorphism. However, the major limitation of our study is the modest sample size, for the interaction analysis. As such, chance can not be ruled out for some of the significant findings. So the interaction of the two established risk factors warrants further investigation in other larger population. Finally, the study was designed to investigate the interaction effect of BPA exposure and LOX pathway polymorphism onto osteosarcoma risk. Since only osteosarcoma patients were included in the study, in such a case, the results may not be generalizable to other tumor diseases.

In conclusion, our study suggests that common genetic variation -22G/C polymorphism in LOX genes may modify the association between BPA exposure and osteosarcoma. The positive results in our study need to be replicated in larger population studies.

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The author(s) declare that they have no competing interests.

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