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Research Paper

Functional Polymorphisms at *ERCC1/XPF* Genes Confer Neuroblastoma Risk in Chinese Children

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ABSTRACT

Variations in nucleotide excision repair pathway genes may predispose to initiation of cancers. However, polymorphisms of *ERCC1/XPF* genes and neuroblastoma risk have not been investigated before. To evaluate the relevance of polymorphisms of *ERCC1/XPF* genes in influencing neuroblastoma susceptibility, we genotyped four polymorphisms in *ERCC1/XPF* genes using a Chinese population of 393 cases and 812 controls. The results showed that *ERCC1* rs2298881 and rs11615 predisposed to enhanced neuroblastoma risk [CA vs. AA: adjusted odds ratio (OR) = 1.94, 95% confidence interval (CI) = 1.30–2.89, $P = 0.0012$; CC vs. AA: adjusted OR = 2.18, 95% CI = 1.45–3.26, $P = 0.0002$ for rs2298881, and AG vs. GG: adjusted OR = 1.31, 95% CI = 1.02–1.69, $P = 0.038$ for rs11615]. Moreover, *XPF* rs2276466 was also associated with increased neuroblastoma risk (GG vs. CC: adjusted OR = 1.66, 95% CI = 1.02–2.71, $P = 0.043$). In the combined analysis of *ERCC1*, we found that carriers with 2–3 risk genotypes were more likely to get risk of neuroblastoma, when compared to those with 0–1 risk genotype (adjusted OR = 1.75; 95% CI = 1.25–2.45, $P = 0.0012$). Our study indicates that common genetic variations in *ERCC1/XPF* genes predispose to neuroblastoma risk, which needs to be further validated by ongoing efforts.

1. Introduction

Neuroblastoma, a heterogeneous tumor developed from neural crest progenitor cells, is the most common solid neoplasm of childhood (Matthay et al., 2016). Neuroblastoma takes up nearly 10% of all childhood cancers, yet its proportion of all pediatric oncology deaths is up to 15% (Cheung and Dyer, 2013). Neuroblastoma is characterized by wide clinical course, with some patients having spontaneous regression without chemotherapy or some having poor prognosis despite intense multi-modal therapy (Maris et al., 2007; Maris, 2010). In general, neuroblastoma cases can be classified into low-, intermediate-, and high-risk groups (Shimada et al., 1999). Nearly 50% of all the neuroblastoma patients are classified into high-risk group, and their survival rates are less than 40% despite intense multi-modal therapy (Matthay et al., 2016). Such unfavorable prognosis was mainly attributed to the extensive metastasis of tumor at the time of diagnosis (Matthay et al., 2016; Esposito et al., 2017).

According to the germline mutations, neuroblastoma is divided into familial and sporadic types. Familial neuroblastoma is rare, with approximately 1–2% of all neuroblastoma cases. The genetic etiology of familial neuroblastoma is relatively elucidated, that is the highly mutations in *PHOX2B* (Mosse et al., 2004; Bourdeaut et al., 2005) or *ALK* gene (Devoto et al., 2011). However, the genetic events predisposing individuals to sporadic neuroblastoma, the most common neuroblastoma, remains unclear. Previous studies indicated that environmental factors such as pregnancy exposures, dwelling condition, and dietary habit are potential risks of sporadic neuroblastoma (Cook et al., 2004; Menegaux et al., 2004; Muller-Schulte et al., 2017), yet there still lacks direct linkage evidence. Mounting evidence has suggested that genetic factors also influence the occurrence of neuroblastoma (Yang et al., 2017; Zhang et al., 2017). For example, common variants of *NEFL* and *CNKN1B* could influence neuroblastoma susceptibility (Capasso et al., 2014; Capasso et al., 2017).

Recent genome-wide association studies (GWASs) have identified genetic variants located in several genes (*HACE1*, *LIN28B*, *BARD1*, *CASC15*, *TP53*, and *LMO1*) associated with neuroblastoma risk by

Abbreviations: GWAS, genome-wide association study; SNP, single nucleotide polymorphism; NER, nucleotide excision repair; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; eQTL, expression quantitative trait loci.

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comparing neuroblastoma patients to healthy controls (Maris et al., 2008; Capasso et al., 2009; Nguyen le et al., 2011; Wang et al., 2011; Diskin et al., 2012; Diskin et al., 2014). Moreover, the role of most of these GWAS-identified single nucleotide polymorphisms (SNPs) in neuroblastoma risk have been confirmed in replication case-control studies (He et al., 2016b; He et al., 2016c; Zhang et al., 2016; He et al., 2017; Zhang et al., 2017). However, these identified genetic variations still account for only a small proportion in predisposing to neuroblastoma.

Therefore, additional gene polymorphisms associated with neuroblastoma susceptibility are needed to be identified. Due to the adoption of the multiple testing correction in the GWAS analysis, some potential SNPs might only have modest risk effects or just be omitted (Stadler et al., 2010). Thus, other research strategies were developed, which include: replication of GWAS-identified SNPs, meta-analysis of GWAS datasets, imputation and epistasis analysis, gene- or pathway-based approaches (Gao, 2011).

In human, DNA repair systems play critical roles in maintaining the stability of cellular functions and genomic integrity (Wood et al., 2001). The nucleotide excision repair (NER) pathway, one of the DNA repair systems, is responsible for excising bulky DNA lesions (Gillet and Scharer, 2006). The NER pathway includes four steps: damage recognition, DNA unwinding, damage excision, and ligation (Friedberg, 2001; Christmann et al., 2003). The eight main members of the NER process, *XPA-XPG* and *XP-V*, are all implicated in maintaining genomic integrity (Cleaver, 2000). The *ERCC1* and *XPF* (also known as *ERCC4*) genes encode proteins that participate in the DNA repair pathways. These two proteins, ERCC1 and XPF, form a heterodimeric complex to cleave the DNA damage on the 5′ side of bubble structures (Sijbers et al., 1996; Evans et al., 1997). Moreover, this complex also functions in the inter-strand crosslink repair (Wood, 2010). Owing to the critical role of ERCC1/XPF complex in maintaining genomic stability, it remains a hot spot of research to explore the role of *ERCC1/XPF* genes variations in cancer risks. To date, epidemiological studies declared that *ERCC1/XPF* genes polymorphisms were associated with cancer risk at different sites, including colorectal cancer (Yang et al., 2015), breast cancer (Yang et al., 2013), gastric cancer (He et al., 2012b), and endometrial cancer (Doherty et al., 2011).

However, the genetic variants driving the *ERCC1/XPF* genetic association with the risk of neuroblastoma has been evaluated in few instances. To determine whether *ERCC1/XPF* genes variations could predispose to neuroblastoma risk or not, we conducted a case-control study in Chinese population.

2. Materials and Methods

2.1. Study Population

This study encompassed 393 cases with neuroblastoma and 812 healthy controls of Chinese origin (He et al., 2018; Zhang et al., 2018). Among them, 275 cases were from Guangzhou Women and Children's Medical Center and 118 were from The First Affiliated Hospital of Zhengzhou University (**Supplemental Table 1**). At the same time, 531 and 281 controls were recruited from the same district, respectively. Additional details and eligibility criteria for subject selection were reported previously (He et al., 2017). All participants or their guardians provided informed consent before the research. The details of the included subjects have been described in our previous publications (He et al., 2016a; Zhang et al., 2017). The study protocols were approved by the Institutional Review Board of Guangzhou Women and Children's Medical Center, and The First Affiliated Hospital of Zhengzhou University.

2.2. SNP Selection and Genotyping

We identified potentially functional SNPs of *ERCC1/XPF* genes from dbSNP database (http://www.ncbi.nlm.nih.gov/) and an online tool, SNPinfo (http://snpinfo.niehs.nih.gov/). Briefly, we searched the potentially functional candidate SNPs located in the 5′- flanking region, 5′ untranslated region, 3′ untranslated region, and exon of *ERCC1/XPF* genes. Additional selection criteria were reported in our previous study (He et al., 2012a). In final, three SNPs (rs2298881, rs3212986, rs11615) with low linkage disequilibrium in the *ERCC1* gene (**Supplemental Fig. 1**, **Supplemental Table 2**) and one SNP (rs2276466) in the *XPF* gene (**Supplemental Table 3**) met the selection criteria. We used TIANamp Blood DNA Kit (TianGen Biotech Co. Ltd., Beijing, China) to extract genomic DNA from peripheral blood donated by subjects. All the selected SNPs were genotyped on a standard commercial TaqMan real-time PCR, with details reported elsewhere (Gong et al., 2017; Li et al., 2017; Lou et al., 2017). As a quality control, eight negative controls with water and eight replicate samples were included in each 384-well plate. Moreover, we randomly selected 10% of the samples to a second run. All duplicate sets had a concordance rate of 100%.

2.3. Statistical Analysis

First, we applied goodness-of-fit χ^2 test to determine whether the selected SNPs among controls were deviated from Hardy-Weinberg equilibrium (HWE). Then we adopted two-sided chi-square test to measure the difference of the demographic variables and allele frequencies between all cases and controls. We also calculated odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression analysis. All statistical analyses were performed using the version 9.4 SAS software (SAS Institute, Cary, NC). All the *P* values were two sided, and *P* values less than 0.05 considered as significant.

2.4. SNP-gene Expression Correlation Analysis

We performed genotype and mRNA expression correlation analysis, using genotyping data from the HapMap phase II release 23 data set and mRNA expression data by genotypes from EBV-transformed B lymphoblastoid cell lines from the same 270 HapMap individuals (He et al., 2012a). We also performed the expression quantitative trait loci (eQTL) analysis using GTEx portal web site (http://www.gtexportal.org/home/) to predict potential associations between the SNPs and gene expression levels (Consortium, 2013).

3. Results

3.1. ERCC1 and XPF Genes Polymorphisms With Neuroblastoma Susceptibility

The detailed characteristics of all the subjects were presented in **Supplemental Table 1** and in our previously published articles (He et al., 2018; Zhang et al., 2018). The distribution of *ERCC1/XPF* genes polymorphisms between all cases and controls were listed in Table 1. In analysis of neuroblastoma patients and controls, three SNPs (two in *ERCC1* and one in *XPF*) were associated with neuroblastoma risk: rs2298881 in *ERCC1* (CA vs. AA: adjusted OR = 1.94, 95% CI = 1.30–2.89, $P = 0.0012$; CC vs. AA: adjusted OR = 2.18, 95% CI = 1.45–3.26, $P = 0.0002$); rs11615 in *ERCC1* (AG vs. GG: adjusted OR = 1.31, 95% CI = 1.02–1.69, $P = 0.038$); and rs2276466 in *XPF* (GG vs. CC: adjusted OR = 1.66, 95% CI = 1.02–2.71, $P = 0.043$). However, we failed to detect a statistically significant relationship between rs3212986 in *ERCC1* and neuroblastoma risk. Higher risk of neuroblastoma was found in individuals with 2–3 combined risk genotypes of *ERCC1*, compared with those with 0–1 risk genotypes (adjusted OR = 1.75; 95% CI = 1.25–2.45, $P = 0.0012$).

3.2. Stratification Analysis

We further evaluated the effects of the selected polymorphisms on the neuroblastoma risk among different strata including age, gender,

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