Association between NER pathway gene polymorphisms and neuroblastoma risk in an eastern Chinese population

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Neuroblastoma is a common childhood malignancy. Nucleotide excision repair (NER) polymorphisms have been shown to influence cancer susceptibility by modifying DNA repair efficiency. To investigate the association of NER gene polymorphisms with neuroblastoma risk, we constructed a three-center case-control study. A total of 19 candidate single-nucleotide polymorphisms (SNPs) in NER genes were analyzed. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the associations. We identified five independent SNPs that were significantly associated with neuroblastoma risk, including XPA rs1800975 (dominant model: adjusted OR = 0.73, 95% CI = 0.55–0.98, p = 0.033), XPA rs3176752 (recessive model: adjusted OR = 2.78, 95% CI = 1.12–6.91, p = 0.028), XPD rs3810366 (dominant: adjusted OR = 1.44, 95% CI = 1.05–1.97, p = 0.022; recessive: adjusted OR = 1.58, 95% CI = 1.18–2.11, p = 0.002), XPD rs238406 (dominant: adjusted OR = 0.64, 95% CI = 0.48–0.84, p = 0.002; recessive: adjusted OR = 0.67, 95% CI = 0.48–0.94, p = 0.021), and XPG rs2094258 (recessive: adjusted OR = 1.44, 95% CI = 1.03–2.04, p = 0.036). Stratified analysis was carried out. Furthermore, these findings were strengthened by false-positive report probability (FPRP) analysis and expression quantitative trait loci (eQTL) analysis. In conclusion, our study indicates that five SNPs in NER genes are correlated with neuroblastoma susceptibility in the eastern Chinese population, providing novel insight into the genetic underpinnings of neuroblastoma. However, further large-scale studies are required to verify these findings.

INTRODUCTION
Neuroblastoma, a common childhood malignancy, arises from the sympathetic nervous system. It mainly occurs in infancy, with a median age of 17 months at diagnosis.1 Additionally, neuroblastoma accounts for about 10% of all malignancies and 15% of malignancy mortality in children.2 Neuroblastoma shows quite a heterogeneity in clinical phenotypes and prognosis. Neuroblastoma patients are generally classified into low-risk, intermediate-risk, and high-risk groups, based on clinical and biological characteristics, including tumor stage, histopathology, age, and MYCN amplification.3–6 Despite significant advances achieved in cancer treatment, the outcome of high-risk neuroblastoma remains poor, with overall survival rates of around 40%.7,8 Therefore, it is necessary to explore the pathogenesis of neuroblastoma and search novel therapies for high-risk neuroblastoma.

Genetic factors play a critical role in neuroblastoma development.9 Genome-wide association studies (GWASs), a powerful tool discovering causal genes and revealing susceptibility variants for diseases,10 have identified some neuroblastoma susceptibility polymorphisms, locating in TP53,11 BARD1,12 HACE1,13 NEFL,14 LMO1,15,16 and LIN28B17 genes. For example, BARD1 rs1048108 and rs17489363 polymorphisms were reported to be associated with neuroblastoma susceptibility.12 Capasso et al.14 also found that NEFL rs1059111 polymorphism could influence neuroblastoma susceptibility by increasing NEFL expression. In addition, Avitabile et al.18 identified that 1p13.2 was a common susceptibility locus for neuroblastoma and melanoma risk by examining pleiotropy across two neural crest cell-derived tumors. Testori et al.19 also identified shared susceptibility loci (locating in BARD1, MSXI, and SHOX2 genes) between two...
neural crest cell originating conditions, that is, neuroblastoma and congenital heart disease. However, intensive investigations are still warranted to uncover additional neuroblastoma susceptibility loci.

DNA repair systems, including base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR), are responsible for maintaining genome integrity and preventing tumorigenesis.20,21

The NER pathway primarily excises bulky DNA lesions.22 Several vital genes are found in the NER pathway, including XPA, XBP/ERCC3, XPC, XPD/ERCC2, XPE/DDB1, XPF/ERCC4, XPG/ERCC5, and ERCC1. Mutations and polymorphisms in NER pathway genes may impair DNA repair ability and therefore increase genome instability.23 Previous investigations have suggested that NER polymorphisms were related to the risk of various cancer types, such as lung cancer,24 breast cancer,25 bladder cancer,26 gastric cancer,27 Wilms tumor,28 and prostate cancer.29 Herein, to determine the roles of NER polymorphisms in neuroblastoma risk, we analyzed 19 candidate SNPs within the NER pathway in 313 neuroblastoma patients and 762 healthy controls from the eastern Chinese population.

RESULTS

Study population

The demographic and clinical characteristics of 313 neuroblastoma patients and 762 controls from the eastern Chinese population are listed in Table S1. Details on participants may be found in previous studies.16,20 There were no significant differences between neuroblastoma patients and healthy controls in age (p = 0.823) and sex (p = 0.610).

Associations of NER pathway gene SNPs with neuroblastoma susceptibility

All of the candidate SNPs were in accordance with the Hardy-Weinberg equilibrium (HWE) in controls. Our findings indicated that five SNPs in NER pathway genes were significantly correlated with neuroblastoma risk, including XPA rs1800975 (dominant model: adjusted odds ratio [OR] = 1.83, 95% confidence interval [CI] = 1.18–2.91, p = 0.003), XPA rs3176752 (recessive model: adjusted OR = 2.78, 95% CI = 1.12–6.91, p = 0.028), XPD rs3810366 (dominant: adjusted OR = 1.44, 95% CI = 1.05–1.97, p = 0.022; recessive: adjusted OR = 1.58, 95% CI = 1.18–2.11, p = 0.002), XPD rs238406 (dominant: adjusted OR = 0.64, 95% CI = 0.48–0.84, p = 0.002; recessive: adjusted OR = 0.67, 95% CI = 0.48–0.94, p = 0.021), and XPG rs2094258 (recessive: adjusted OR = 1.44, 95% CI = 1.03–2.04, p = 0.036) polymorphisms (Table 1).

Stratified analysis

Furthermore, stratified analysis by age, sex, and tumor sites was performed for significant SNPs and the combined risk genotypes. The

Table 1. Association of polymorphisms in nucleotide excision repair pathway genes with neuroblastoma susceptibility in eastern Chinese children

| Gene | SNP | Case (N = 313) | Control (N = 762) |
|------|-----|---------------|------------------|
|      | Allele | A | B | AA | AB | BB | AOR (95% CI) | p<sup>a</sup> | AOR (95% CI) | p<sup>b</sup> | HWE |
|      |       |   |   |    |    |    |   |  |   |  |   |
| ERCC1| rs2298881 | C | A | 117 | 156 | 40 | 298 | 347 | 116 | 1.09 (0.83–1.43) | 0.529 | 0.83 (0.56–1.22) | 0.346 | 0.367 |
| ERCC1| rs3121986 | C | A | 157 | 131 | 25 | 356 | 323 | 82 | 0.87 (0.67–1.13) | 0.304 | 0.73 (0.45–1.16) | 0.179 | 0.496 |
| ERCC1| rs11615 | G | A | 168 | 118 | 27 | 441 | 270 | 50 | 1.18 (0.91–1.54) | 0.214 | 1.32 (0.81–2.16) | 0.263 | 0.322 |
| XPA | rs1800975 | T | C | 100 | 142 | 71 | 196 | 382 | 184 | 0.73 (0.55–0.98) | 0.033 | 0.92 (0.67–1.26) | 0.611 | 0.937 |
| XPA | rs3176752 | G | T | 237 | 66 | 10 | 589 | 164 | 9 | 1.10 (0.80–1.49) | 0.564 | 2.78 (1.12–6.91) | 0.028 | 0.520 |
| XPC | rs2228001 | A | C | 127 | 150 | 36 | 309 | 350 | 103 | 1.00 (0.77–1.31) | 0.981 | 0.84 (0.56–1.26) | 0.400 | 0.805 |
| XPC | rs2228000 | C | T | 145 | 123 | 45 | 351 | 330 | 81 | 0.99 (0.76–1.29) | 0.917 | 1.41 (0.95–2.09) | 0.085 | 0.793 |
| XPC | rs2607775 | C | G | 289 | 24 | 0 | 696 | 63 | 3 | 0.89 (0.54–1.44) | 0.624 | / | / | 0.228 |
| XPC | rs1870134 | G | C | 182 | 114 | 17 | 418 | 291 | 53 | 0.87 (0.67–1.14) | 0.306 | 0.75 (0.43–1.32) | 0.314 | 0.808 |
| XPC | rs2229090 | G | C | 123 | 136 | 54 | 316 | 339 | 107 | 1.09 (0.83–1.42) | 0.540 | 1.28 (0.89–1.83) | 0.179 | 0.296 |
| XPD | rs3810366 | G | C | 67 | 145 | 101 | 213 | 371 | 177 | 1.44 (1.05–1.97) | 0.022 | 1.58 (1.18–2.11) | 0.002 | 0.530 |
| XPD | rs238406 | G | T | 116 | 142 | 55 | 208 | 371 | 182 | 0.64 (0.48–0.84) | 0.002 | 0.67 (0.48–0.94) | 0.021 | 0.511 |
| XPD | rs13181 | T | G | 249 | 62 | 62 | 635 | 117 | 9 | 1.28 (0.92–1.79) | 0.149 | 0.55 (0.12–2.58) | 0.448 | 0.177 |
| XPF | rs2276466 | C | G | 204 | 94 | 15 | 488 | 238 | 35 | 0.95 (0.72–1.26) | 0.735 | 1.83 (0.55–1.91) | 0.931 | 0.389 |
| XPG | rs2094258 | C | T | 114 | 135 | 62 | 310 | 340 | 112 | 1.19 (0.90–1.56) | 0.217 | 1.44 (1.03–2.04) | 0.036 | 0.235 |
| XPG | rs751402 | C | T | 148 | 130 | 33 | 317 | 371 | 74 | 0.79 (0.60–1.03) | 0.076 | 1.10 (0.71–1.69) | 0.681 | 0.020 |
| XPG | rs2296147 | T | C | 197 | 99 | 15 | 467 | 269 | 26 | 0.90 (0.69–1.19) | 0.468 | 1.42 (0.74–2.72) | 0.292 | 0.089 |
| XPG | rs1047768 | T | C | 163 | 120 | 28 | 395 | 314 | 55 | 0.97 (0.74–1.26) | 0.810 | 1.33 (0.82–2.14) | 0.249 | 0.376 |
| XPG | rs873601 | G | A | 84 | 168 | 59 | 204 | 376 | 182 | 0.98 (0.73–1.32) | 0.906 | 0.74 (0.53–1.03) | 0.073 | 0.734 |

SNP, single-nucleotide polymorphism; AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

<sup>a</sup>Adjusted for age and sex for dominant model.

<sup>b</sup>Adjusted for age and sex for recessive model.
XPA rs1800975 TC/CC was shown to significantly reduce neuroblastoma risk in children >18 months of age and in subgroups with tumors originating from the adrenal gland/mediastinum. The XPA rs3176752 TT was shown to significantly increase neuroblastoma risk in children >18 months of age, boys, and in subgroups with tumors originating from the adrenal gland/mediastinum. In the combined analysis, we observed that carriers with one to two risk genotypes of XPA had a significantly increased neuroblastoma risk in children >18 months of age and in subgroups with tumors originating from the adrenal gland/mediastinum, compared to non-carriers (Table 2).

The XPD rs3810366 GC/CC significantly increased neuroblastoma risk in children ≤18 months of age, boys, and in subgroups with tumors originating from the retroperitoneum. The XPD rs238406 GT/TT conferred reduced neuroblastoma risk in children ≤18 months of age and in subgroups with females/males in which tumor originated from the mediastinum. In the combined analysis, we observed that carriers with two to three risk genotypes of XPD exhibited a significantly increased neuroblastoma risk in children ≤18 months of age, boys, and in subgroups with tumors originating from retroperitoneum/mediastinum, compared to those with no risk and one risk genotypes (Table 3).

Individuals with the XPG rs2094258 TT genotype tended to develop neuroblastoma in the retroperitoneum. In the combined analysis, we observed that carriers with one to five risk genotypes of XPG showed a significantly increased neuroblastoma risk in children >18 months of age, and in subgroups with females/males, compared to non-carriers (Table 4).

False-positive report probability (FPRP) analysis
We further calculated the FPRP values for all significant genetic effects observed in our study. As shown in Table 5, we preset 0.2 as the FPRP threshold at the prior probability of 0.1. The significant association for the XPD rs3810366 G>C genotype remained noteworthy (FPRP = 0.052) in the overall analysis, as well as in a stratified analysis (FPRP = 0.038 in children ≤18 months of age). The association for the XPD rs238406 G>T genotype was noteworthy in the whole study population (FPRP = 0.039), as well as in children ≤18 months of age (FPRP = 0.037). Moreover, in the combined analysis, the associations for two to three risk genotypes of the XPD gene were still noteworthy in children ≤18 months of age (FPRP = 0.048).

Expression quantitative trait loci (eQTL) analysis
We further explored biological effects of the five significant SNPs on gene expressions by eQTL analysis from the genotype-tissue expression (GTEx) portal. We observed that the TSTD2 mRNA level with the rs1800975 C genotype was significantly higher than those with the rs1800975 T genotype in the tibial nerve (Figure 1). We also found that both of two SNPs (rs3810366 and rs238406) were correlated with the mRNA levels of PPP1R13L and XPD/ERCC2 genes (Figure 2). Additionally, the METT21EP mRNA level with the rs2094258 C genotype was significantly higher than those with the rs2094258 T genotype in the tibial nerve and cell-cultured fibroblasts (Figure 3).

DISCUSSION
Neuroblastoma is the most common extracranial solid tumor among children. Genetic aberrations play an important role in neuroblastoma. The NER pathway is the primary mechanism of DNA repair pathways, which plays an essential role in maintaining genomic stability and preventing tumorigenesis. Polymorphisms in NER genes resulting in variation of DNA repair efficiency have been shown to influence the risk of cancer development.

To systematically explore the potential associations between NER polymorphisms and neuroblastoma risk in the eastern Chinese
induced by both exogenous and endogenous factors. Several critical factors, including SNPs in six core NER genes, were analyzed. Our data suggested that carriers with specific SNPs had no statistical differences in subgroups, which might indicate that mutations in the XPA, XPD, and XPG genes have been reported to play essential roles in the NER process. The XPA gene, encoding a DNA-binding protein, is involved in the NER pathway to maintain genomic integrity by interacting with other NER proteins. Current evidence indicates that mutations in XPA may impair the DNA repair ability and lead to increased cancer risk. Zienolddiny et al. found that XPA rs1800975 was significantly related to the risk of lung cancer. The XPD gene encodes an evolutionarily conserved ATP-dependent helicase, which functions in basal transcription and NER. The XPD polymorphisms have been reported to be associated with cancer risk, such as nasopharyngeal carcinoma, renal cell carcinoma, and esophageal cancer.

The NER pathway is an essential mechanism to remove DNA damage induced by both exogenous and endogenous factors. Several critical genes (e.g., XPA, XPD, and XPG) have been reported to play essential roles in the NER process. The XPA gene, encoding a DNA-binding protein, is involved in the NER pathway to maintain genomic integrity by interacting with other NER proteins. Current evidence indicates that mutations in XPA may impair the DNA repair ability and lead to increased cancer risk. Zienolddiny et al. found that XPA rs1800975 was significantly related to the risk of lung cancer. The XPD gene encodes an evolutionarily conserved ATP-dependent helicase, which functions in basal transcription and NER. The XPD polymorphisms have been reported to be associated with cancer risk, such as nasopharyngeal carcinoma, renal cell carcinoma, and esophageal cancer.

Table 3. Stratification analysis for the association of XPD genotypes and neuroblastoma susceptibility in eastern Chinese children

| Variables     | rs3810366 (case/control) | rs238406 (case/control) | Risk genotype (case/control) |
|---------------|--------------------------|-------------------------|-----------------------------|
|               | GG                        | GC/CC                   | AOR (95% CI)                | p*          | 0–1 | 2–3 | AOR (95% CI) | p*          |
| Age (months)  | GG                        | GC/CC                   | AOR (95% CI)                | p*          | 0–1 | 2–3 | AOR (95% CI) | p*          |
| <18           | 19/111                    | 123/229                 | 3.15 (1.85–5.38)            | <0.0001     | 12/93 | 130/247 | 4.10 (2.16–7.76) | <0.0001     |
| >18           | 48/102                    | 123/319                 | 0.82 (0.55–1.22)            | 0.320       | 55/123 | 116/298 | 0.87 (0.59–1.28) | 0.486       |
| Sex           | Female                    | 37/95                   | 1.14 (0.73–1.78)            | 0.563       | 51/89 | 94/250 | 0.65 (0.43–0.99) | 0.044       |
|               | Male                      | 30/118                  | 1.78 (1.14–2.79)            | 0.012       | 65/119 | 103/330 | 0.62 (0.43–0.91) | 0.013       |
| Sites of origin | Adrenal gland             | 18/213                  | 1.10 (0.63–1.93)            | 0.738       | 25/208 | 43/553 | 0.64 (0.38–1.08) | 0.095       |
|               | Retropertitoneum           | 24/213                  | 1.67 (1.04–2.69)            | 0.033       | 44/208 | 82/553 | 0.69 (0.46–1.03) | 0.072       |
|               | Mediastinum               | 19/213                  | 1.66 (0.98–2.82)            | 0.058       | 40/208 | 59/553 | 0.55 (0.36–0.85) | 0.007       |
|               | Others                    | 6/213                   | 0.97 (0.37–2.58)            | 0.958       | 7/208 | 13/553 | 0.67 (0.26–1.71) | 0.399       |

aAdjusted for age and sex, omitting the corresponding stratification factor.
bRisk genotypes were carriers with rs3810366 GC/CC, rs13181 TT/TG, and rs238406 GT/GG genotypes.

discussion, we carried out a three-center case-control study with 313 neuroblastoma cases and 762 healthy controls. Overall, 19 candidate SNPs in six core NER genes were analyzed. Our data suggested that five SNPs were significantly correlated with the risk of neuroblastoma, including XPA (rs1800975 and rs3176752), XPD (rs3810366 and rs238406), and XPG rs2094258 polymorphisms. Some candidate SNPs had no statistical differences in subgroups, which might suggest that the small sample size in the stratified analysis.

The NER pathway is an essential mechanism to remove DNA damage induced by both exogenous and endogenous factors. Several critical genes (e.g., XPA, XPD, and XPG) have been reported to play essential roles in the NER process. The XPA gene, encoding a DNA-binding protein, is involved in the NER pathway to maintain genomic integrity by interacting with other NER proteins. Current evidence indicates that mutations in XPA may impair the DNA repair ability and lead to increased cancer risk. Zienolddiny et al. found that XPA rs1800975 was significantly related to the risk of lung cancer. The XPD gene encodes an evolutionarily conserved ATP-dependent helicase, which functions in basal transcription and NER. The XPD polymorphisms have been reported to be associated with cancer risk, such as nasopharyngeal carcinoma, renal cell carcinoma, and esophageal cancer.

Table 4. Stratification analysis for the association of XPG genotypes and neuroblastoma susceptibility in eastern Chinese children

| Variables     | rs2094258 (case/control) | rs873601 (case/control) | Risk genotype (case/control) |
|---------------|--------------------------|-------------------------|-----------------------------|
|               | CC/CT                    | TT                      | AOR (95% CI)                | p*          | 0–1 | 2–5 | AOR (95% CI) | p*          |
| Age (months)  | CC/CT                    | TT                      | AOR (95% CI)                | p*          | 0–1 | 2–5 | AOR (95% CI) | p*          |
| <18           | 11/290                   | 30/50                   | 1.57 (0.95–2.60)            | 0.079       | 110/257 | 31/83 | 0.87 (0.54–1.38) | 0.546       |
| >18           | 138/360                  | 32/62                   | 1.36 (0.85–2.17)            | 0.204       | 142/323 | 28/99 | 0.65 (0.41–1.03) | 0.066       |
| Sex           | Female                   | 112/288                 | 1.63 (0.99–2.67)            | 0.055       | 113/254 | 31/86 | 0.82 (0.51–1.32) | 0.416       |
|               | Male                     | 137/362                 | 1.32 (0.82–2.14)            | 0.253       | 139/326 | 28/96 | 0.69 (0.43–1.10) | 0.116       |
| Sites of origin | Adrenal gland            | 58/650                  | 0.91 (0.44–1.88)            | 0.793       | 52/580 | 15/182 | 0.92 (0.51–1.67) | 0.784       |
|               | Retropertitoneum         | 96/650                  | 1.81 (1.14–2.85)            | 0.011       | 103/580 | 23/182 | 0.71 (0.44–1.15) | 0.158       |
|               | Mediastinum              | 77/650                  | 1.59 (0.94–2.68)            | 0.084       | 81/580 | 17/182 | 0.66 (0.38–1.15) | 0.144       |
|               | Others                   | 18/650                  | 0.66 (0.15–2.87)            | 0.576       | 16/580 | 4/182 | 0.79 (0.26–3.9)  | 0.670       |

aAdjusted for age and sex, omitting the corresponding stratification factor.
bRisk genotypes were carriers with rs2094258 CT/TT, rs751402 CC, rs2996147 CC, rs1047768 CC, and rs873601 GA/GG genotypes.
| Genotype | Crude OR (95% CI) | p<sup>a</sup> | Statistical power<sup>b</sup> | Prior probability |
|----------|------------------|-----------|-----------------|------------------|
|          |                  |           |                 | 0.25  | 0.1   | 0.01  | 0.001  | 0.0001 |
| XPA rs180975T>C |                  |           |                 |       |       |       |       |       |
| TC/CC versus TT | 0.74 (0.55–0.98) | 0.038     | 0.749           | 0.133 | 0.314 | 0.835 | 0.981 | 0.998 |
| >18 months of age | 0.64 (0.43–0.94) | 0.023     | 0.402           | 0.147 | 0.340 | 0.850 | 0.983 | 0.998 |
| Adrenal gland | 0.56 (0.33–0.94) | 0.027     | 0.253           | 0.244 | 0.492 | 0.914 | 0.991 | 0.999 |
| Mediastinum | 0.63 (0.41–0.99) | 0.043     | 0.405           | 0.242 | 0.490 | 0.913 | 0.991 | 0.999 |
| XPA rs3176752G>T |                |           |                 |       |       |       |       |       |
| GG versus GT/TT | 2.76 (1.11–6.86) | 0.029     | 0.093           | 0.483 | 0.737 | 0.969 | 0.997 | 1.000 |
| >18 months of age | 4.46 (1.29–15.49) | 0.018     | 0.043           | 0.559 | 0.792 | 0.977 | 0.998 | 1.000 |
| Males | 5.17 (1.28–20.93) | 0.021     | 0.042           | 0.599 | 0.818 | 0.980 | 0.998 | 1.000 |
| Adrenal gland | 3.86 (1.02–14.62) | 0.047     | 0.091           | 0.606 | 0.822 | 0.981 | 0.998 | 1.000 |
| Mediastinum | 4.45 (1.46–13.56) | 0.009     | 0.033           | 0.439 | 0.702 | 0.963 | 0.996 | 1.000 |
| XPA rs3176752G>T |                 |           |                 |       |       |       |       |       |
| XPA 1–2 versus 0 risk genotypes |                |           |                 |       |       |       |       |       |
| >18 months of age | 1.57 (1.06–2.31) | 0.023     | 0.412           | 0.143 | 0.334 | 0.847 | 0.982 | 0.998 |
| Adrenal gland | 1.78 (1.16–2.97) | 0.029     | 0.267           | 0.246 | 0.494 | 0.915 | 0.991 | 0.999 |
| Mediastinum | 1.57 (1.01–2.44) | 0.046     | 0.426           | 0.246 | 0.495 | 0.915 | 0.991 | 0.999 |
| XPD rs3810366G>C |                |           |                 |       |       |       |       |       |
| GG versus GC/CC | 1.57 (1.18–2.10) | 0.002     | 0.379           | 0.018 | 0.052 | 0.376 | 0.859 | 0.984 |
| GC/GG versus CC | 1.43 (1.04–1.95) | 0.026     | 0.621           | 0.112 | 0.274 | 0.806 | 0.977 | 0.998 |
| ≤18 months of age | 3.14 (1.84–5.35) | <0.0001 | 0.006           | 0.013 | 0.038 | 0.302 | 0.813 | 0.978 |
| Males | 1.79 (1.140–2.80) | 0.011     | 0.238           | 0.125 | 0.299 | 0.825 | 0.979 | 0.998 |
| Retropertioneum | 1.65 (1.03–2.65) | 0.037     | 0.354           | 0.239 | 0.486 | 0.912 | 0.991 | 0.999 |
| XPD rs3810366G>C |                |           |                 |       |       |       |       |       |
| XPD 2–3 versus 0 risk genotypes |                |           |                 |       |       |       |       |       |
| ≤18 months of age | 4.08 (2.16–7.71) | <0.0001 | 0.003           | 0.016 | 0.048 | 0.355 | 0.847 | 0.982 |
| Males | 2.06 (1.25–3.40) | 0.005     | 0.125           | 0.101 | 0.253 | 0.788 | 0.974 | 0.997 |
| Retropertioneum | 2.05 (1.20–3.50) | 0.009     | 0.145           | 0.159 | 0.361 | 0.862 | 0.984 | 0.998 |
| Mediastinum | 2.31 (1.24–4.32) | 0.009     | 0.103           | 0.203 | 0.433 | 0.893 | 0.988 | 0.999 |
| XPG rs2094258C>T |                |           |                 |       |       |       |       |       |
| TT versus CT/CC | 1.45 (1.03–2.04) | 0.035     | 0.590           | 0.152 | 0.350 | 0.856 | 0.984 | 0.998 |
| Retropertioneum | 1.81 (1.15–2.86) | 0.011     | 0.211           | 0.131 | 0.311 | 0.832 | 0.980 | 0.998 |
| XPG 1–5 versus 0 risk genotypes |                |           |                 |       |       |       |       |       |
| >18 months of age | 2.27 (1.30–3.95) | 0.004     | 0.090           | 0.112 | 0.275 | 0.807 | 0.977 | 0.998 |
| Females | 2.19 (1.24–3.85) | 0.007     | 0.115           | 0.151 | 0.348 | 0.854 | 0.983 | 0.998 |
| Males | 1.85 (1.07–3.20) | 0.028     | 0.246           | 0.255 | 0.507 | 0.919 | 0.991 | 0.999 |
| Retropertioneum | 2.20 (1.21–4.01) | 0.010     | 0.122           | 0.198 | 0.425 | 0.891 | 0.988 | 0.999 |
| Mediastinum | 2.23 (1.13–4.39) | 0.020     | 0.142           | 0.301 | 0.563 | 0.934 | 0.993 | 0.999 |

<sup>a</sup>Chi-square test was used to calculate the genotype frequency distributions.

<sup>b</sup>Statistical power was calculated using the number of observations in the subgroup and the OR and p values in this table.
squamous cell carcinoma, and breast cancer. Zhu et al. reported that the XPD (rs3810366 and rs238406) polymorphisms contributed significantly to the risk of Wilms tumor. Zhao et al. also found that XPD rs238406 was significantly associated with the increased risk of ovarian cancer. The XPG gene encodes a structure-specific endonuclease, which also plays a vital role in the NER pathway. The XPG protein could stabilize the DNA repair complex of damaged DNA by excising damaged oligonucleotide during the NER process. Our previous study also found that XPG rs2094258 was significantly related to the risk of neuroblastoma in a Chinese population. Therefore, it suggests that the functional SNPs in XPA, XPD, and XPG correlate with cancer risk by influencing the ability of DNA repair.

A single NER polymorphism may have a limited effect on neuroblastoma risk. Indeed, we explored the impact of several risk genotypes of neuroblastoma by combination analysis. The combined analyses in subgroups showed that patients, carrying combined risk genotypes of NER pathway genes, had significantly increased neuroblastoma risk in individuals when compared with those with a no risk or one risk genotype. The result indicated that the combined NER polymorphisms had a much stronger effect on neuroblastoma susceptibility than did the single one. Additionally, the eQTL analysis revealed that significant SNPs also affected the expressions of local or distant genes in human tissues.

There were several limitations to our study. First, the statistical power may be limited due to the relatively small sample size. Second, due to the retrospective study, selection and information bias might be unavoidable. Third, the polymorphisms were restricted to unrelated Han Chinese, and the findings may not be applicable to other ethnicities. Fourth, although 19 candidate SNPs in six core genes were analyzed in the present study, more potentially functional NER polymorphisms were needed to be investigated. Finally, biological experiments should be performed to further confirm the findings of eQTL analysis.

In conclusion, our findings reveal that the five significant SNPs (XPA rs1800975 and rs3176752, XPD rs3810366 and rs238406, and XPG rs2094258) may contribute to neuroblastoma risk in an eastern Chinese population, and they provide potential genetic markers for the prediction of neuroblastoma susceptibility. However, large-scale studies are required to verify these findings, and the false discovery rate (FDR) or Bonferroni corrections are needed to correct multiple testing in the future.

MATERIALS AND METHODS

Study population
Participants were recruited from three independent hospitals as follows: Children’s Hospital of Nanjing Medical University (158 neuroblastoma patients and 426 healthy controls, Jiangsu Province, China),
Anhui Provincial Children’s Hospital (119 neuroblastoma patients and 264 healthy controls), Anhui Province, China, and The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University (36 neuroblastoma patients and 72 healthy controls, Wenzhou, China). A total of 313 neuroblastoma patients and 762 healthy controls from the eastern Chinese population were included in the case-control study. All participants were unrelated Chinese Han children. The study was approved by each participating hospital Institutional Review Board. The selection standard and details of the included participants were accessible in our previous studies. Written informed consent was obtained from all participants or their guardians before the study.

SNP selection and genotyping
The potentially functional SNPs were selected from the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) from several perspectives: (1) location of SNPs in the gene region, (2) minor allele frequency, and (3) linkage disequilibrium (LD). The SNPinfo database (http://snpinfo.niehs.nih.gov/snpfunc.html) was used to predict the potential function of SNPs (such as altering amino acids, affecting the binding ability of transcription factors or microRNA binding sites). We ultimately chose 19 candidate SNPs from six core NER pathway genes according to the previous selection criteria. As shown in Table S2, there was no significant LD (R² < 0.8) among most of these 19 SNPs. However, there was a moderate LD between rs238406 and rs3810366 (R² = 0.856) and between rs2229090 and rs2228000 (R² = 0.875).

DNA samples were extracted as previously described. Genotyping was performed using TaqMan real-time PCR on the ABI 7900 genetic detection system. The details of the genotyping protocol were described in a previous study. Quality control was strictly performed; duplicate negative controls and positive controls were included on each plate. Additionally, 10% of the samples were randomly chosen for duplicate analyses. The concordance of genotyping results was confirmed.

eQTL analysis
eQTL are loci or markers on the genomes, which are associated with gene expressions. The GTEx project (https://www.gtexportal.org/home/index.html) aims to evaluate the relationship between genetic variation and gene expressions in normal human tissues. We explored the influences of significant SNPs on gene expressions in tibial nerve or cell-cultured fibroblasts by eQTL analysis from the GTEx portal. Details on the aim, design, and data analysis of the study were described in the previous study.

Statistical analysis
HWE in controls was performed using a goodness-of-fit χ² test. Differences in the categorical variables between cases and controls were assessed using the χ² test. Logistic regression was conducted with adjustment for age and sex. ORs and 95% CIs were used to evaluate the association between the polymorphisms and the risk of neuroblastoma. We further performed the FPRP analysis to assess whether the significant findings were noteworthy. The prior probability of 0.1 was adopted to detect the noteworthiness for OR. The significant results with FPRP <0.2 were considered noteworthy. All statistical tests were performed with SAS software (v9.1; SAS Institute, Cary, NC, USA). A two-sided p value of <0.05 was considered statistically significant, without extra notification.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.omto.2020.12.004.

ACKNOWLEDGMENTS
This work was supported by grants from the National Natural Science Foundation of China (nos. 81900529 and 81502046) and from the Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease (no. 2019B030301004).

AUTHOR CONTRIBUTIONS
J.H. and H.W. designed the experiments, supervised the project, and were involved in all aspects of the submission. C.Z., Y.W., L.H., J.Z., J.L., Y.T., H.Z., and J.H. performed the experiments and participated in the study design, data analysis, and manuscript preparation. All authors reviewed and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.
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