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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% confidential 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 *LIN28B*[17] 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*, *MSX1*, and *SHOX2* genes) between two

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

		Allele		Case (N = 313)			Control (N = 762)							
Gene	SNP	A	B	AA	AB	BB	AA	AB	BB	AOR (95% CI)[a]	p[a]	AOR (95% CI)[b]	p[b]	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	rs3212986	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	2	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	1.03 (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	53	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.
[a]Adjusted for age and sex for dominant model.
[b]Adjusted for age and sex for recessive model.

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*, *XPB*/*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.[16,30] 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] = 0.73, 95% confidence interval [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) 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 2. Stratification analysis for the association of *XPA* genotypes and neuroblastoma susceptibility in eastern Chinese children

	rs1800975 (case/control)				rs3176752 (case/control)				Risk genotypes[b] (case/control)			
Variables	TT	TC/CC	AOR (95% CI)[a]	p[a]	GG/GT	TT	AOR (95% CI)[a]	p[a]	0	1–2	AOR (95% CI)[a]	p[a]
Age (months)												
≤18	43/94	99/246	0.86 (0.56–1.33)	0.495	139/335	3/5	1.46 (0.34–6.20)	0.610	99/245	43/95	1.14 (0.74–1.76)	0.544
>18	57/102	114/320	0.64 (0.43–0.95)	0.025	164/418	7/4	4.53 (1.31–15.71)	0.017	114/320	57/102	1.56 (1.06–2.31)	0.025
Sex												
Female	42/73	103/267	0.66 (0.42–1.03)	0.065	141/334	4/6	1.72 (0.47–6.28)	0.409	103/266	42/74	1.49 (0.96–2.33)	0.079
Male	58/123	110/299	0.78 (0.53–1.14)	0.199	162/419	6/3	5.22 (1.29–21.11)	0.021	110/299	58/123	1.28 (0.88–1.88)	0.199
Sites of origin												
Adrenal gland	26/196	42/566	0.56 (0.34–0.95)	0.030	65/753	3/9	3.85 (1.01–14.68)	0.048	42/565	26/197	1.76 (1.05–2.96)	0.032
Retroperitoneum	34/196	92/566	0.92 (0.60–1.41)	0.690	125/753	1/9	0.64 (0.08–5.07)	0.670	92/565	34/197	1.08 (0.71–1.66)	0.718
Mediastinum	35/196	64/566	0.63 (0.40–0.98)	0.041	94/753	5/9	4.46 (1.46–13.60)	0.009	64/565	35/197	1.58 (1.01–2.46)	0.044
Others	5/196	15/566	1.07 (0.38–3.00)	0.897	19/753	1/9	4.51 (0.53–38.18)	0.167	15/565	5/197	0.93 (0.33–2.60)	0.884

[a]Adjusted for age and sex, omitting the corresponding stratification factor.
[b]Risk genotypes were carriers with rs1800975 CC and rs3176752 TT genotypes.

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* rs3810366G>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* rs238406G>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.[31] 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.[20] Polymorphisms in NER genes resulting in variation of DNA repair efficiency have been shown to influence the risk of cancer development.[32]

To systemically explore the potential associations between NER polymorphisms and neuroblastoma risk in the eastern Chinese

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

	rs3810366 (case/control)				rs238406 (case/control)				Risk genotype[b] (case/control)			
Variables	GG	GC/CC	AOR (95% CI)[a]	p[a]	GG	GT/TT	AOR (95% CI)[a]	p[a]	0–1	2–3	AOR (95% CI)[a]	p[a]
Age (months)												
≤18	19/111	123/229	3.15 (1.85–5.38)	<0.0001	61/85	81/255	0.44 (0.29–0.66)	<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	37/91	134/330	0.99 (0.65–1.53)	0.979
Sex												
Female	37/95	108/244	1.14 (0.73–1.78)	0.563	51/89	94/250	0.65 (0.43–0.99)	0.044	27/84	118/255	1.44 (0.88–2.34)	0.148
Male	30/118	138/304	1.78 (1.14–2.79)	0.012	65/119	103/330	0.62 (0.43–0.91)	0.013	22/100	146/322	2.06 (1.25–3.40)	0.005
Sites of origin												
Adrenal gland	18/213	50/548	1.10 (0.63–1.93)	0.738	25/208	43/553	0.64 (0.38–1.08)	0.095	16/184	52/577	1.05 (0.59–1.89)	0.870
Retroperitoneum	24/213	102/548	1.67 (1.04–2.69)	0.033	44/208	82/553	0.69 (0.46–1.03)	0.072	17/184	109/577	2.08 (1.21–3.56)	0.008
Mediastinum	19/213	80/548	1.66 (0.98–2.82)	0.058	40/208	59/553	0.55 (0.36–0.85)	0.007	12/184	87/577	2.35 (1.25–4.39)	0.008
Others	6/213	14/548	0.97 (0.37–2.58)	0.958	7/208	13/553	0.67 (0.26–1.71)	0.399	4/184	16/577	1.36 (0.45–4.12)	0.593

[a]Adjusted for age and sex, omitting the corresponding stratification factor.
[b]Risk genotypes were carriers with rs3810366 GC/CC, rs13181 TT/TG, and rs238406 GT/GG genotypes.

population, 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 due to 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.[20,32] 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 increase cancer risk.[33,34] Zienolddiny et al.[24] 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,[35] renal cell carcinoma,[36] esophageal

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

	rs2094258 (case/control)				rs873601 (case/control)				Risk genotype[b] (case/control)			
Variables	CC/CT	TT	AOR (95% CI)[a]	p[a]	GG/GA	AA	AOR (95% CI)[a]	p[a]	0	1–5	AOR (95% CI)[a]	p[a]
Age (months)												
≤18	111/290	30/50	1.57 (0.95–2.60)	0.079	110/257	31/83	0.87 (0.54–1.38)	0.546	18/69	123/271	1.74 (0.99–3.05)	0.054
>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	17/85	153/337	2.26 (1.30–3.94)	0.004
Sex												
Female	112/288	32/52	1.63 (0.99–2.67)	0.055	113/254	31/86	0.82 (0.51–1.32)	0.416	17/77	127/263	2.11 (1.19–3.73)	0.010
Male	137/362	30/60	1.32 (0.82–2.14)	0.253	139/326	28/96	0.69 (0.43–1.10)	0.116	18/77	149/345	1.84 (1.06–3.18)	0.030
Sites of origin												
Adrenal gland	58/650	9/112	0.91 (0.44–1.88)	0.793	52/580	15/182	0.92 (0.51–1.67)	0.784	10/154	57/608	1.43 (0.71–2.87)	0.311
Retroperitoneum	96/650	30/112	1.81 (1.14–2.85)	0.011	103/580	23/182	0.71 (0.44–1.15)	0.158	13/154	113/608	2.23 (1.22–4.06)	0.009
Mediastinum	77/650	21/112	1.59 (0.94–2.68)	0.084	81/580	17/182	0.66 (0.38–1.15)	0.144	10/154	88/608	2.24 (1.14–4.41)	0.020
Others	18/650	2/112	0.66 (0.15–2.87)	0.576	16/580	4/182	0.79 (0.26–2.39)	0.670	2/154	18/608	2.28 (0.52–9.95)	0.273

[a]Adjusted for age and sex, omitting the corresponding stratification factor.
[b]Risk genotypes were carriers with rs2094258 CT/TT, rs751402 CC, rs2296147 CC, rs1047768 CC, and rs873601 GA/GG genotypes.

Table 5. False-positive report probability analysis for the significant findings

Genotype	Crude OR (95% CI)	p[a]	Statistical power[b]	Prior probability				
				0.25	0.1	0.01	0.001	0.0001
XPA rs1800975T>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 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 (1.06–2.97)	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
Retroperitoneum	1.65 (1.03–2.65)	0.037	0.354	0.239	0.486	0.912	0.991	0.999
XPD rs238406G>T								
GG versus GT/TT	0.68 (0.49–0.95)	0.023	0.531	0.116	0.283	0.813	0.978	0.998
GT/GG versus TT	0.64 (0.48–0.85)	0.002	0.373	0.013	0.039	0.311	0.820	0.979
≤18 months of age	0.44 (0.29–0.67)	0.0001	0.024	0.013	0.037	0.295	0.809	0.977
Females	0.66 (0.43–0.997)	0.048	0.463	0.238	0.484	0.912	0.990	0.999
Males	0.62 (0.43–0.91)	0.013	0.300	0.118	0.286	0.815	0.978	0.998
Mediastinum	0.56 (0.36–0.86)	0.008	0.202	0.100	0.251	0.786	0.974	0.997
XPD 2–3 versus 0–1 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
Retroperitoneum	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
Retroperitoneum	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
Retroperitoneum	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

[a]Chi-square test was used to calculate the genotype frequency distributions.
[b]Statistical power was calculated using the number of observations in the subgroup and the OR and p values in this table.

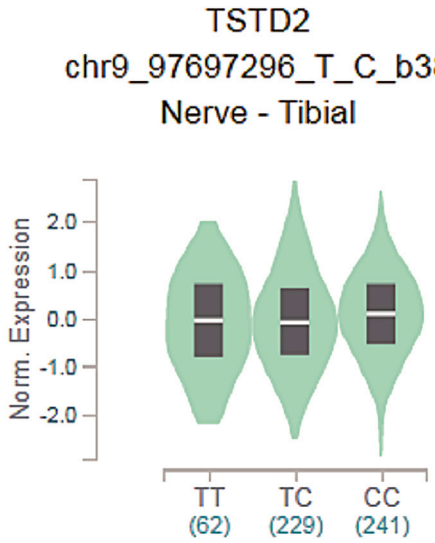

Figure 1. Functional prediction of the rs1800975 polymorphism in the tibial nerve

Genotype-based mRNA expression alteration in the tibial nerve for the *XPA* rs1800975T>C polymorphism using data from the GTEx portal database ($p = 1.8 \times 10^{-5}$).

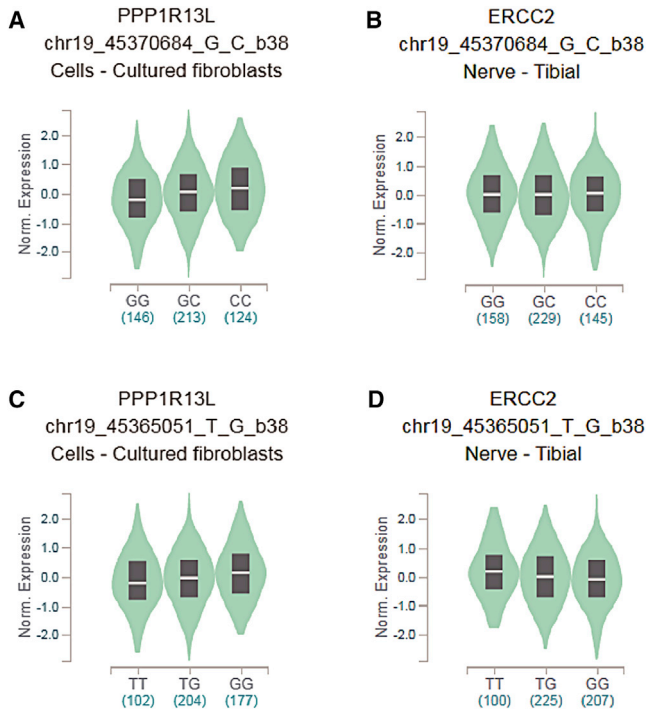

Figure 2. Functional prediction of the rs3810366 and rs238406 polymorphisms in cell-cultured fibroblasts and the tibial nerve

(A–D) Genotype-based mRNA expression alteration for the *XPD* rs3810366G>C polymorphism in (A) cell-cultured fibroblasts ($p = 2.3 \times 10^{-10}$) and (B) the tibial nerve ($p = 3.5 \times 10^{-8}$) and for the *XPD* rs238406G>T polymorphism in (C) cell-cultured fibroblasts ($p = 2.3 \times 10^{-8}$) and (D) the tibial nerve ($p = 8.1 \times 10^{-3}$), using data from the GTEx portal database.

squamous cell carcinoma,[37] and breast cancer.[38] Zhu et al. reported that the *XPD* (rs3810366 and rs238406) polymorphisms contributed significantly to the risk of Wilms tumor.[28] Zhao et al.[39] 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.[40–42] Our previous study also found that *XPG* rs2094258 was significantly related to the risk of neuroblastoma in a Chinese population.[43] 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),

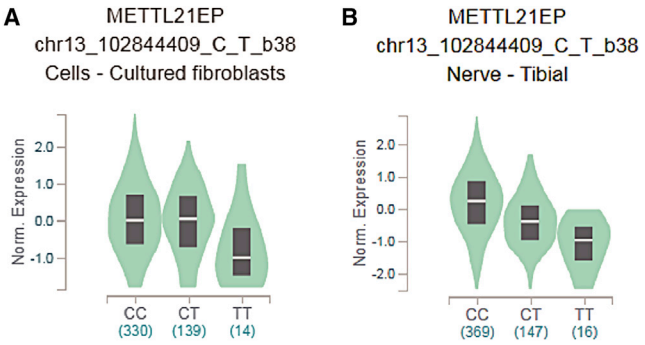

Figure 3. Functional prediction of the rs2094258 polymorphism in cell-cultured fibroblasts and the tibial nerve

(A and B) Genotype-based mRNA expression alteration for *XPG* rs2094258C>T polymorphism in (A) cell-cultured fibroblasts ($p = 1.7 \times 10^{-14}$) and (B) the tibial nerve ($p = 3.0 \times 10^{-6}$), using data from the GTEx portal database.

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.[16,30,44] 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.[16] 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.[28] As shown in Table S2, there was no significant LD ($R^2 < 0.8$) among most of these 19 SNPs. However, there was a moderate LD between rs238406 and rs3810366 ($R^2 = 0.856$) and between rs2229090 and rs2228000 ($R^2 = 0.875$).

DNA samples were extracted as previously described.[43] 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.[28] 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.[45] 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.[46]

Statistical analysis

HWE in controls was performed using a goodness-of-fit χ^2 test. Differences in the categorical variables between cases and controls were assessed using the χ^2 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.[47,48] 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.

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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.

REFERENCES

1. London, W.B., Castleberry, R.P., Matthay, K.K., Look, A.T., Seeger, R.C., Shimada, H., Thorner, P., Brodeur, G., Maris, J.M., Reynolds, C.P., and Cohn, S.L. (2005). Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. J. Clin. Oncol. *23*, 6459–6465.
2. Maris, J.M., Hogarty, M.D., Bagatell, R., and Cohn, S.L. (2007). Neuroblastoma. Lancet *369*, 2106–2120.
3. Maris, J.M. (2010). Recent advances in neuroblastoma. N. Engl. J. Med. *362*, 2202–2211.
4. Matthay, K.K., Maris, J.M., Schleiermacher, G., Nakagawara, A., Mackall, C.L., Diller, L., and Weiss, W.A. (2016). Neuroblastoma. Nat. Rev. Dis. Primers *2*, 16078.

5. Irwin, M.S., and Park, J.R. (2015). Neuroblastoma: paradigm for precision medicine. Pediatr. Clin. North Am. *62*, 225–256.

6. Tanimoto, T., Tazawa, H., Ieda, T., Nouso, H., Tani, M., Oyama, T., Urata, Y., Kagawa, S., Noda, T., and Fujiwara, T. (2020). Elimination of MYCN-amplified neuroblastoma cells by telomerase-targeted oncolytic virus via MYCN suppression. Mol. Ther. Oncolytics *18*, 14–23.

7. Spix, C., Pastore, G., Sankila, R., Stiller, C.A., and Steliarova-Foucher, E. (2006). Neuroblastoma incidence and survival in European children (1978–1997): report from the Automated Childhood Cancer Information System project. Eur. J. Cancer *42*, 2081–2091.

8. Mahapatra, S., and Challagundla, K.B. (2020). Neuroblastoma. StatPearls (StatPearls Publishing).

9. Schwab, M., Westermann, F., Hero, B., and Berthold, F. (2003). Neuroblastoma: biology and molecular and chromosomal pathology. Lancet Oncol. *4*, 472–480.

10. De, R., Bush, W.S., and Moore, J.H. (2014). Bioinformatics challenges in genome-wide association studies (GWAS). Methods Mol. Biol. *1168*, 63–81.

11. He, J., Wang, F., Zhu, J., Zhang, Z., Zou, Y., Zhang, R., Yang, T., and Xia, H. (2017). The *TP53* gene rs1042522 C>G polymorphism and neuroblastoma risk in Chinese children. Aging (Albany NY) *9*, 852–859.

12. Cimmino, F., Avitabile, M., Diskin, S.J., Vaksman, Z., Pignataro, P., Formicola, D., Cardinale, A., Testori, A., Koster, J., de Torres, C., et al. (2018). Fine mapping of 2q35 high-risk neuroblastoma locus reveals independent functional risk variants and suggests full-length BARD1 as tumor-suppressor. Int. J. Cancer *143*, 2828–2837.

13. Diskin, S.J., Capasso, M., Schnepp, R.W., Cole, K.A., Attiyeh, E.F., Hou, C., Diamond, M., Carpenter, E.L., Winter, C., Lee, H., et al. (2012). Common variation at 6q16 within *HACE1* and *LIN28B* influences susceptibility to neuroblastoma. Nat. Genet. *44*, 1126–1130.

14. Capasso, M., Diskin, S., Cimmino, F., Acierno, G., Totaro, F., Petrosino, G., Pezone, L., Diamond, M., McDaniel, L., Hakonarson, H., et al. (2014). Common genetic variants in NEFL influence gene expression and neuroblastoma risk. Cancer Res. *74*, 6913–6924.

15. Wang, K., Diskin, S.J., Zhang, H., Attiyeh, E.F., Winter, C., Hou, C., Schnepp, R.W., Diamond, M., Bosse, K., Mayes, P.A., et al. (2011). Integrative genomics identifies *LMO1* as a neuroblastoma oncogene. Nature *469*, 216–220.

16. He, L., Zhu, J., Han, F., Tang, Y., Zhou, C., Dai, J., Wang, Y., Zhou, H., He, J., and Wu, H. (2018). *LMO1* gene polymorphisms reduce neuroblastoma risk in eastern Chinese children: a three-center case-control study. Front. Oncol. *8*, 468.

17. He, J., Yang, T., Zhang, R., Zhu, J., Wang, F., Zou, Y., and Xia, H. (2016). Potentially functional polymorphisms in the *LIN28B* gene contribute to neuroblastoma susceptibility in Chinese children. J. Cell. Mol. Med. *20*, 1534–1541.

18. Avitabile, M., Succoio, M., Testori, A., Cardinale, A., Vaksman, Z., Lasorsa, V.A., Cantalupo, S., Esposito, M., Cimmino, F., Montella, A., et al. (2020). Neural crest-derived tumor neuroblastoma and melanoma share 1p13.2 as susceptibility locus that shows a long-range interaction with the *SLC16A1* gene. Carcinogenesis *41*, 284–295.

19. Testori, A., Lasorsa, V.A., Cimmino, F., Cantalupo, S., Cardinale, A., Avitabile, M., Limongelli, G., Russo, M.G., Diskin, S., Maris, J., et al. (2019). Exploring shared susceptibility between two neural crest cells originating conditions: neuroblastoma and congenital heart disease. Genes (Basel) *10*, 663.

20. Wood, R.D., Mitchell, M., Sgouros, J., and Lindahl, T. (2001). Human DNA repair genes. Science *291*, 1284–1289.

21. Sancar, A. (1995). DNA repair in humans. Annu. Rev. Genet. *29*, 69–105.

22. Friedberg, E.C. (2001). How nucleotide excision repair protects against cancer. Nat. Rev. Cancer *1*, 22–33.

23. Kamileri, I., Karakasilioti, I., and Garinis, G.A. (2012). Nucleotide excision repair: new tricks with old bricks. Trends Genet. *28*, 566–573.

24. Zienolddiny, S., Campa, D., Lind, H., Ryberg, D., Skaug, V., Stangeland, L., Phillips, D.H., Canzian, F., and Haugen, A. (2006). Polymorphisms of DNA repair genes and risk of non-small cell lung cancer. Carcinogenesis *27*, 560–567.

25. He, B.S., Xu, T., Pan, Y.Q., Wang, H.J., Cho, W.C., Lin, K., Sun, H.L., Gao, T.Y., and Wang, S.K. (2016). Nucleotide excision repair pathway gene polymorphisms are linked to breast cancer risk in a Chinese population. Oncotarget *7*, 84872–84882.

26. García-Closas, M., Malats, N., Real, F.X., Welch, R., Kogevinas, M., Chatterjee, N., Pfeiffer, R., Silverman, D., Dosemeci, M., Tardón, A., et al. (2006). Genetic variation in the nucleotide excision repair pathway and bladder cancer risk. Cancer Epidemiol. Biomarkers Prev. *15*, 536–542.

27. He, J., Zhuo, Z.J., Zhang, A., Zhu, J., Hua, R.X., Xue, W.Q., Zhang, S.D., Zhang, J.B., Li, X.Z., and Jia, W.H. (2018). Genetic variants in the nucleotide excision repair pathway genes and gastric cancer susceptibility in a southern Chinese population. Cancer Manag. Res. *10*, 765–774.

28. Zhu, J., Fu, W., Jia, W., Xia, H., Liu, G.C., and He, J. (2018). Association between NER pathway gene polymorphisms and Wilms tumor risk. Mol. Ther. Nucleic Acids *12*, 854–860.

29. Wang, M., Li, Q., Gu, C., Zhu, Y., Yang, Y., Wang, J., Jin, L., He, J., Ye, D., and Wei, Q. (2017). Polymorphisms in nucleotide excision repair genes and risk of primary prostate cancer in Chinese Han populations. Oncotarget *8*, 24362–24371.

30. Zhuo, Z., Zhou, C., Fang, Y., Zhu, J., Lu, H., Zhou, H., Wu, H., Wang, Y., and He, J. (2020). Correlation between the genetic variants of base excision repair (BER) pathway genes and neuroblastoma susceptibility in eastern Chinese children. Cancer Commun. (Lond.) *40*, 641–646.

31. Tonini, G.P. (2019). Neuroblastoma by chance. J. Cancer *10*, 2601–2603.

32. Al-Shaheri, F.N., Al-Shami, K.M., Gamal, E.H., Mahasneh, A.A., and Ayoub, N.M. (2020). Association of DNA repair gene polymorphisms with colorectal cancer risk and treatment outcomes. Exp. Mol. Pathol. *113*, 104364.

33. States, J.C., McDuffie, E.R., Myrand, S.P., McDowell, M., and Cleaver, J.E. (1998). Distribution of mutations in the human xeroderma pigmentosum group A gene and their relationships to the functional regions of the DNA damage recognition protein. Hum. Mutat. *12*, 103–113.

34. Ikegami, T., Kuraoka, I., Saijo, M., Kodo, N., Kyogoku, Y., Morikawa, K., Tanaka, K., and Shirakawa, M. (1998). Solution structure of the DNA- and RPA-binding domain of the human repair factor XPA. Nat. Struct. Biol. *5*, 701–706.

35. Ban, E.Z., Lye, M.S., Chong, P.P., Yap, Y.Y., Lim, S.Y.C., and Abdul Rahman, H. (2017). Haplotype CGC from XPD, hOGG1 and ITGA2 polymorphisms increases the risk of nasopharyngeal carcinoma in Malaysia. PLoS ONE *12*, e0187200.

36. Loghin, A., Bănescu, C., Nechifor-Boila, A., Chibelean, C., Orsolya, M., Nechifor-Boila, A., Tripon, F., Voidazan, S., and Borda, A. (2016). *XRCC3* Thr241Met and *XPD* Lys751Gln gene polymorphisms and risk of clear cell renal cell carcinoma. Cancer Biomark. *16*, 211–217.

37. Zhu, M.L., He, J., Wang, M., Sun, M.H., Jin, L., Wang, X., Yang, Y.J., Wang, J.C., Zheng, L., Xiang, J.Q., and Wei, Q.Y. (2014). Potentially functional polymorphisms in the *ERCC2* gene and risk of esophageal squamous cell carcinoma in Chinese populations. Sci. Rep. *4*, 6281.

38. Shore, R.E., Zeleniuch-Jacquotte, A., Currie, D., Mohrenweiser, H., Afanasyeva, Y., Koenig, K.L., Arslan, A.A., Toniolo, P., and Wirgin, I. (2008). Polymorphisms in *XPC* and *ERCC2* genes, smoking and breast cancer risk. Int. J. Cancer *122*, 2101–2105.

39. Zhao, Z., Zhang, A., Zhao, Y., Xiang, J., Yu, D., Liang, Z., Xu, C., Zhang, Q., Li, J., and Duan, P. (2018). The association of polymorphisms in nucleotide excision repair genes with ovarian cancer susceptibility. Biosci. Rep. *38*, BSR20180114.

40. Wakasugi, M., Reardon, J.T., and Sancar, A. (1997). The non-catalytic function of XPG protein during dual incision in human nucleotide excision repair. J. Biol. Chem. *272*, 16030–16034.

41. O'Donovan, A., Davies, A.A., Moggs, J.G., West, S.C., and Wood, R.D. (1994). XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. Nature *371*, 432–435.

42. Friedberg, E.C. (2003). DNA damage and repair. Nature *421*, 436–440.

43. He, J., Wang, F., Zhu, J., Zhang, R., Yang, T., Zou, Y., and Xia, H. (2016). Association of potentially functional variants in the *XPG* gene with neuroblastoma risk in a Chinese population. J. Cell. Mol. Med. *20*, 1481–1490.

44. Zhou, C., Tang, Y., Zhu, J., He, L., Li, J., Wang, Y., Zhou, H., He, J., and Wu, H. (2019). Association of *miR-146a*, *miR-149* and *miR-196a2* polymorphisms with

neuroblastoma risk in eastern Chinese population: a three-center case-control study. Biosci. Rep. *39*, BSR20181907.

45. Zhuo, Z.J., Liu, W., Zhang, J., Zhu, J., Zhang, R., Tang, J., Yang, T., Zou, Y., He, J., and Xia, H. (2018). Functional polymorphisms at ERCC1/XPF genes confer neuroblastoma risk in Chinese children. EBioMedicine *30*, 113–119.

46. GTEx Consortium (2013). The Genotype-Tissue Expression (GTEx) project. Nat. Genet. *45*, 580–585.

47. Wacholder, S., Chanock, S., Garcia-Closas, M., El Ghormli, L., and Rothman, N. (2004). Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J. Natl. Cancer Inst. *96*, 434–442.

48. He, J., Wang, M.Y., Qiu, L.X., Zhu, M.L., Shi, T.Y., Zhou, X.Y., Sun, M.H., Yang, Y.J., Wang, J.C., Jin, L., et al. (2013). Genetic variations of mTORC1 genes and risk of gastric cancer in an eastern Chinese population. Mol. Carcinog. *52* (*Suppl 1*), E70–E79.