Abstract

Neuroblastoma is a pediatric malignancy arising from the developing peripheral nervous system. p53 and downstream effector miR-34b/c have critical tumor suppressing functions. TP53 Arg72Pro (rs1042522 C > G) and miR-34b/c rs4938723 (T > C) polymorphisms have been known to modify cancer susceptibility. This study was performed to validate the association of these two polymorphisms and neuroblastoma risk with 819 cases and 1780 controls. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were used to assess the strength of the associations. False positive report possibility analysis was adopted to dissect out real significant associations from chance findings. We found that both TP53 Arg72Pro (CG/GG vs. CC: adjusted OR = 0.82, 95% CI = 0.69-0.98) and miR-34b/c rs4938723 (TC/CC vs. TT: adjusted OR = 0.64, 95% CI = 0.54-0.75) were associated with decreased neuroblastoma susceptibility. Stratify analyses further confirmed the protective effect among some subgroups. Moreover, subjects with variant alleles of both polymorphisms were associated with more significantly decreased neuroblastoma risk (CG/TC vs. CC/TT: adjusted OR = 0.38, 95% CI = 0.28-0.50; GG/TC vs. CC/TT: adjusted OR = 0.43, 95% CI = 0.30-0.63) than those carrying variant allele of either one polymorphism (CC/TC vs. CC/TT: adjusted OR = 0.51, 95%
Neuroblastoma is an extracranial neuroendocrine tumor, affecting approximately 25 to 50 individuals per million [1]. Tumor may occur in the adrenal glands and/or sympathetic ganglia. The majority of tumors (90%) are diagnosed in children younger than 10 years old, and the median age at diagnosis is about 18 months old. Neuroblastoma is a group of heterogeneous diseases. Its clinical presentation and prognosis vary greatly dependent on the tumor biology, including molecular genetics. Neuroblastoma is also a complex genetic disease [2–4]. Apart from driver gene mutations [2], genome-wide association studies (GWAS) have identified a number of neuroblastoma susceptibility loci in the CASC15, BARD1, DUSP12, DDX4, IL31RA, HSD17B12, LMO1, HACE1, LIN28B, MLF1, and C7Z genes [5–9]. As a complementary to agnostic approach, traditional candidate gene method is also frequently used to investigate genetic variation in protein coding sequences. Recently, studies by candidate gene approaches have found some genetic variations associated with neuroblastoma risk in Chinese children by performing this case control study with 819 cases and 1780 controls.

Materials and Methods

Study Population

Only patients with newly diagnosed and histopathologically confirmed neuroblastoma were qualified to be recruited for this study. Healthy controls were frequency-matched to cases on the basis of age and gender. Totally, 819 controls and 1780 cases were separately recruited from seven hospitals in China, including Hunan Children’s Hospital (162 cases and 270 controls), Guangzhou Women and Children’s Medical Center (275 cases and 531 controls) [27,28], the Second Affiliated Hospital of Xi’an Jiaotong University (76 cases and 186 controls) [29], The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University (36 cases and 72 controls) [30], The First Affiliated Hospital of Zhengzhou University (118 cases and 281 controls) [31,32], Children’s Hospital of Shanxi (33 cases and 176 controls), and Anhui Provincial Children’s Hospital (119 cases and 264 controls) [33]. Written informed consent was obtained from all participants or their guardians. The institutional review boards of Guangzhou Women and Children’s Medical Center, the First Affiliated Hospital of Zhengzhou University, Anhui Provincial Children’s Hospital, Hunan Children’s Hospital, the Second Affiliated Hospital of Xi’an Jiaotong University, Children Hospital and Women Health Center of Shanxi, The First Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University authorized this study.

SNP Selection and Genotyping

Two potentially functional SNPs were chosen for this study based on previous publications [23,28,34]. The $TP53$ Arg72Pro (rs1042522 C > G) is a common nonsynonymous SNP, generating two biochemically and biologically polymorphic variants, $p53$ Arg and $p53$ Pro [19]. The rs4938723 T > C polymorphism is located in the promoter region of pri-miR-34b/c, which was initially reported to be associated with an elevated risk of developing primary hepatocellular carcinoma [23]. Genomic DNA was isolated from venous blood samples donated by participants, use the TIANamp Genomic DNA blood kit (Tiangen Biotech, Beijing, China). Allelic discrimination TaqMan assay was employed to genotype SNPs in 384-wellplates with strict quality control [35–39]. Assay was run in the ABI 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). Individuals involved in genotyping remain blind to status of blood donor.

Introduction

Neuroblastoma is a neuroendocrine tumor, affecting approximately 25 to 50 individuals per million [1]. Tumor may occur in the adrenal glands and/or sympathetic ganglia. The majority of tumors (90%) are diagnosed in children younger than 10 years old, and the median age at diagnosis is about 18 months old. Neuroblastoma is a group of heterogeneous diseases. Its clinical presentation and prognosis vary greatly dependent on the tumor biology, including molecular genetics. Neuroblastoma is also a complex genetic disease [2–4]. Apart from driver gene mutations [2], genome-wide association studies (GWAS) have identified a number of neuroblastoma susceptibility loci in the CASC15, BARD1, DUSP12, DDX4, IL31RA, HSD17B12, LMO1, HACE1, LIN28B, MLF1, and C7Z genes [5–9]. As a complementary to agnostic approach, traditional candidate gene method is also frequently used to investigate genetic variation in protein coding sequences. Recently, studies by candidate gene approaches have found some genetic variations associated with neuroblastoma risk in Chinese children by performing this case control study with 819 cases and 1780 controls.

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Clinical stages

Table 2. Associations Between TP53 and miR-34b/c Polymorphisms and Neuroblastoma Susceptibility

| Genotype | Cases (N = 819) | Controls (N = 1780) | P1 | Crude OR (95% CI) | P | Adjusted OR (95% CI) | P1 |
|----------|----------------|---------------------|----|------------------|---|---------------------|----|
| TP53 rs1042522 C>G (HWE = 0.541)
| CC | 285 (34.80) | 544 (30.58) | 1.00 |                      | 1.00 |                      | 1.00 |
| CG | 375 (45.79) | 891 (50.08) | 0.80 (0.67–0.97) | .022 | 0.80 (0.67–0.97) | .022 |
| GG | 159 (19.41) | 344 (19.34) | 0.88 (0.70–1.12) | .299 | 0.88 (0.69–1.11) | .285 |
| Additive | | | | | | | |
| Dominant | 534 (65.20) | 1235 (69.62) | 0.52 | 0.92 (0.82–1.04) | .164 | 0.92 (0.82–1.03) | .156 |
| Recessive | 660 (80.59) | 1435 (80.66) | 0.965 | 1.01 (0.82–1.24) | .965 | 1.00 (0.81–1.24) | .988 |
| C | 945 (75.69) | 1979 (55.62) | 1.00 |                      | 1.00 |                      | 1.00 |
| G | 693 (42.31) | 1579 (44.38) | 0.92 (0.82–1.03) | .162 | 0.92 (0.82–1.03) | .153 |
| miR-34b/c rs4938723 T>C (HWE = 0.276)
| TT | 455 (56.66) | 808 (45.44) | 1.00 |                      | 1.00 |                      | 1.00 |
| TC | 242 (30.14) | 796 (44.77) | 0.54 (0.45–0.65) | <.0001 | 0.54 (0.45–0.65) | <.0001 |
| CC | 106 (13.20) | 174 (9.79) | 1.08 (0.83–1.41) | 1.564 | 1.08 (0.83–1.41) | 0.578 |
| Additive | | | | | | | |
| Dominant | 348 (43.34) | 970 (54.56) | <.0001 | 0.84 (0.74–0.95) | .006 | 0.84 (0.74–0.95) | .006 |
| Recessive | 697 (86.80) | 1604 (90.21) | .010 | 1.40 (1.08–1.81) | .010 | 1.40 (1.08–1.81) | .011 |
| T | 1152 (71.73) | 2412 (67.83) | 1.00 |                      | 1.00 |                      | 1.00 |
| C | 454 (28.27) | 1144 (32.17) | 0.83 (0.73–0.95) | .005 | 0.83 (0.73–0.95) | .005 |

OR, odds ratio; CI, confidence interval.
1 $\chi^2$ test for genotype distributions between neuroblastoma cases and cancer-free controls.
2 Adjusted for age and gender.
3 There were missing values for genotyping that failed.

Statistical Analysis

Frequency distributions of demographic variables and genotype were compared between cases and controls using $\chi^2$ test. Hardy–Weinberg equilibrium (HWE) was checked for the frequency distribution of target SNPs among control subjects, using the goodness-of-chi-squared test. Unconditional logistic regression was used to generate odds ratios (ORs) and 95% confidence intervals (CIs) in order to estimate the association of studied polymorphisms with neuroblastoma risk. OR and 95% CI were estimated under Hardy–Weinberg equilibrium (HWE) $\chi^2$ test.

Results

Association of miR-34b/c rs4938723 and TP53 Arg72Pro Polymorphisms with Neuroblastoma Susceptibility

No significant difference was detected between cases and controls for age ($P = .395$) and gender ($P = .832$) for combined subjects (Supplemental Table 1). Both of the two studied SNPs were shown to exert protective effects against neuroblastoma (Table 1). TP53 rs1042522 C>G polymorphism was associated with decreased findings. As indicated by previous publication [40], we used a prior probability of 0.1 to interrogate OR of 1.50/0.67 (risk/protective association) with the significance level of FPRP predetermined as 0.2. The association with a FPRP value of <0.2 was considered noteworthy. All statistical analyses were two-sided and carried out using SAS software (Version 9.1; SAS Institute, Cary, NC, USA). A significance level of $P < .05$ was applied without extra specification.

Table 2. Stratification Analysis of TP53 and miR-34b/c Polymorphisms with Neuroblastoma Susceptibility

| Variables | rs1042522 (cases/controls) | AOR (95% CI) | P1 | rs4938723 (cases/controls) | AOR (95% CI) | P1 |
|-----------|-----------------------------|--------------|----|-----------------------------|--------------|----|
| Age, month | | | | | | | |
| ≤18 | 110 (232) | 216 (508) | 0.89 (0.67–1.17) | .393 | 181 (334) | 139 (406) | 0.63 (0.48–0.82) | .0006 |
| >18 | 175 (312) | 318 (727) | 0.78 (0.62–0.97) | .029 | 274 (474) | 209 (564) | 0.64 (0.52–0.80) | <.0001 |
| Gender | | | | | | | |
| Females | 122 (241) | 235 (526) | 0.87 (0.67–1.14) | .304 | 210 (342) | 142 (425) | 0.54 (0.42–0.70) | <.0001 |
| Males | 163 (303) | 299 (799) | 0.79 (0.62–0.99) | .043 | 245 (466) | 206 (545) | 0.72 (0.58–0.90) | .004 |
| Sites of origin | | | | | | | |
| Adrenal gland | 90 (544) | 168 (1235) | 0.82 (0.62–1.08) | .316 | 160 (808) | 97 (797) | 0.51 (0.39–0.66) | <.0001 |
| Retroperitoneal | 93 (344) | 188 (1235) | 0.88 (0.68–1.16) | .360 | 154 (808) | 117 (797) | 0.63 (0.49–0.82) | <.0005 |
| Mediastinum | 80 (544) | 123 (1235) | 0.68 (0.51–0.92) | .012 | 98 (808) | 102 (970) | 0.87 (0.65–1.16) | .341 |
| Others | 20 (544) | 49 (1235) | 1.09 (0.64–1.85) | .759 | 39 (808) | 29 (970) | 0.62 (0.38–1.01) | .056 |
| Clinical stages | | | | | | | |
| I + II + IV | 154 (544) | 288 (1235) | 0.83 (0.66–1.03) | .089 | 258 (808) | 200 (970) | 0.70 (0.57–0.86) | <.0009 |
| III + IV | 121 (544) | 231 (1235) | 0.84 (0.66–1.07) | .147 | 204 (808) | 139 (970) | 0.57 (0.45–0.72) | <.0001 |

AOR, adjusted odds ratio; CI, confidence interval.
1 Adjusted for age and gender, omitting the corresponding stratify factor.
neuroblastoma susceptibility [CG vs. CC adjusted OR (AOR) = 0.80, 95% CI = 0.67-0.97; CG/GG vs.CC: AOR = 0.82, 95% CI = 0.69-0.98]. miR-34b/c rs4938723 T > C polymorphism also conferred reduced neuroblastoma susceptibility (TC vs. TT, AOR = 0.54, 95% CI = 0.45-0.65; additive model: AOR = 0.84, 95% CI = 0.74-0.95; TC/CC vs. TT: AOR = 0.64, 95% CI = 0.54-0.75; CC vs. TC/TT: AOR = 1.40, 95% CI = 1.08-1.81; C vs. T: AOR = 0.83, 95% CI = 0.73-0.95).

**Stratification Analysis**

We further performed stratification analysis to dissect the effects of confounding factors on the strength of the association, including age, gender, sites of origin and clinical stages (Table 2). Regarding the protective effect of TP53 rs1042522 CG/GG genotypes, significant association resided in children old than 18 months (AOR = 0.78, 95% CI = 0.62-0.97), males (AOR = 0.79, 95% CI = 0.62-0.99), and those with tumors in mediastinum (AOR = 0.68, 95% CI = 0.51-0.92). There was no modification of this result by clinical stages. In contrast, the protective effect of the miR-34b/c rs4938723 TC/CC genotypes remained significant among all subgroups, except for strata with tumor in mediastinum and “others”.

**Combined Effect Analysis**

To explore the combined effect of SNPs in miR-34b/c and TP53 gene, we tested the association between inferred genotype combinations and neuroblastoma susceptibility (Table 3). The following genotype combinations were shown to decrease susceptibility to neuroblastoma when compared to combination of wide type genotype (CC/TC vs. CC/TT: AOR = 0.51, 95% CI = 0.37-0.69; CG/TT vs. CC/TT: AOR = 0.71, 95% CI = 0.55-0.92; CG/TC vs. CC/TT: AOR = 0.38, 95% CI = 0.28-0.50; GG/TC vs. CC/TT: AOR = 0.43, 95% CI = 0.30-0.63). However, the carriers of
combination of variant genotype (GG/CC) did not show significantly
decreased risk probably because of small sample size (AOR = 0.91,
95% CI = 0.52-1.60). Furthermore, we found that subjects with
variant alleles of both polymorphisms have smaller ORs (0.38 for
CG/TC; 0.43 for GG/TC) than those carrying variant allele of either
one polymorphism (0.51 for CC/TC; 0.71 for CG/TT). It suggests
that combined protective effects conferred by two SNPs are stronger
than either one alone and the former are less likely to develop
neuroblastoma than the latter.

False Positive Report Possibility Analysis

The results of association studies are often questioned by false
positivity. To address this issue, the FPRP analysis was performed to
test the credibility of our significant findings (Table 4). FPRP analysis
determines whether a statistically significant finding is noteworthy by
collectively considering statistical power of the study, the calculated P
value, and the prior probability of reality of the association, which is
more objective than statistical significance based on a P < .05 alone
[40]. With a prior probability of 0.25, all our significant findings are
deserving of attention. While probability was lowered to 0.1, all tested
results remained noteworthy, except for the association under the
dominant model and the association for older children and males for
rs1042522 C > G polymorphism. When the standard of prior
probability become more strict (0.001), results for rs1042522 C > G
polymorphism became not deserving of attention, but most of results
for rs4938723 T > C maintained to be noteworthy, even with much
smaller prior possibilities. It suggests that latter is higher penetrant
SNP than the former. Overall, FPRP analysis confirmed the
credibility of our results.

Discussion

The importance of p53 in tumor suppression can be partially reflected
by the fact genetic alterations (e.g., mutations) in the p53 signaling
pathway are implicated in nearly all types of human cancers. In
response to DNA damage, cellular stress, and excessive mitogenic
stimulation, p53 is activated to trigger apoptosis, cellular senescence
or cell cycle arrest to maintain homeostasis [16,41]. As a component
of p53 tumor suppression network, human pri-miR-34a and pri-miR-34b/c
genes are mapped to Chr.1p36 and Chr.11q23 [42]. Mechanistic study revealed that pri-miR-34b/pri-miR-34c can inhibit proliferation of
ovarian cancer cell [43], miR-34 also acts as a tumor suppressor in
neuroblastoma by targeting MYCN [44] and CD44 [45], suggesting
the implication of miR-34 family in neuroblastoma. mRNA expression profiling analysis revealed that miR-34 suppressed cell
cycle genes of neuroblastoma IMR32 cells [44], miR-34 also induced
apoptosis of neuroblastoma cells and inhibited DNA synthesis [44].
miR-34b/c is processed from a common primary transcript (pri-miR-34b/c).
In response to stimuli (e.g., DNA damage), p53 promotes the expression of miR-34 by transcriptionally activating the
miRNA-encoding gene; miR-34 in turn induced cell cycle arrest by
facilitating the degradation of transcripts of target genes including
CCNE2, CDK4 and the MET [16]. Therefore, miR-34 is important
downstream effectors of p53 signaling cascades [16,41]. Epigenetic
inactivation of miR-34 gene by CpG methylation has been observed
in several types of cancer [42].

We have previously explored the association between TP53 gene
rs1042522 C > G polymorphism and neuroblastoma susceptibility in
Chinese children, with 256 patients and 531 controls [34]. Because
the sample size was relatively small, the association only reached
borderline significant (CG vs. CC: OR = 0.72, 95% CI = 0.51-1.02,
P = .065) [34]. Diskin et al. reported that the association of TP53
gene rs35850753 and rs7837822 polymorphisms and susceptibility
to neuroblastoma [45]. However, Cattelan et al. found lack of
association between minor allele of TP53 rs1042522 and neuro-
blastoma risk in an Italy population with 288 healthy subjects and
286 neuroblastoma patients. Alternatively, the same study revealed
significant association between minor allele of rs1042522 and poor
neuroblastoma prognosis [46], validating the role of this SNP in the
neuroblastoma. It is not uncommon to generate conflicting results for
observational association case-control studies. Association results
could be also affected by sample size, sampling strategy, genotyping
method, geographic region, and ethnicity.

miR-34b/c rs4938723 has been reported to be associated with the
risk of a wide spectrum of cancer, including esophageal squamous cell
carcinoma, childhood acute lymphoblastic leukemia, hepatocellular
carcinoma, gastric cancer, and prostate cancer [26,47–53]. With a
study population of 393 cases and 812 controls, we for the first time
reported a protective association between the miR-34b/c rs4938723
and neuroblastoma risk [28].

In this study, we aimed to validate our findings above and evaluate
combined effects of these two SNPs on neuroblastoma risk in a larger
study. In the current study, the triple sample size of 819 cases and
1780 controls allowed us to detect significant association between
TP53 rs1042522 C > G polymorphism and neuroblastoma suscepti-
bility under the heterogeneous and dominant model. Moreover, the
association of the miR-34b/c rs4938723 with neuroblastoma risk was
validated in this study. These two SNPs may exert protective effects
cumulatively. We found that subjects with variant alleles of both
polymorphisms are less likely to develop neuroblastoma than those
carrying variant allele of either one polymorphism. FPRP analysis
indicated that most of our significant findings are noteworthy with a
prior probability of 0.1.

There is evidence indicating that the TP53 Arg72Pro polymorph-
ism may affect the function of p53 [17–19,54]. For instance, this
nonsynonymous common SNP not only changed the primary
structure of the protein, but also led to differential migration rate
during sodium dodecyl sulfate polyacrylamide gel electrophoresis
[17]. A study showed that the exogenous p53 Arg was significantly
more vulnerable than p53 Pro to the ubiquitin-mediated degradation
in p53-null Saos-2 cells when exposed to human papillomavirus
(HPV) E6 protein [18]. Moreover, p53 Arg and p53 Pro differed in
term of their abilities to transcriptionally activate target genes, to
induce apoptosis, and to suppress the transformation of primary
murine fibroblasts [19]. However, the underlying molecular
mechanisms for its association with reduced neuroblastoma suscepti-
bility need to be clarified.

Recently, two meta-analyses revealed that the roles of the miR-34b/c
rs4938723 in cancer susceptibility are tissue dependent [20,55].
The rs4938723 polymorphism was shown to significantly increase
the risk of hepatocellular carcinoma but decreased the risk of
developing esophageal squamous cell carcinoma, colorectal cancer,
and acute lymphoblastic leukemia. Several possibilities may help to
explain such conflicting situation. This T to C transition poly-
morphism is positioned in the promoter region of pri-miR-34b/c,
within a typical CpG island specifically. According to bioinformatics
analysis, this SNP may affect predicted GATA-X transcription
factors’ binding to the promoter of pri-miR-34b/c gene so as to alter
its expression levels. Given transcription factors regulate gene
expression in a tissue-specific way, this SNP may affect different transcription factors’ binding to the promoter, thereby either upregulating or downregulating transcription in different tissues. Moreover, the same microRNA may target different genes in the different tissues, and thereby modify cancer susceptibility in the tissue-specific manner.

This study also has limitations to be addressed. First, only two functional SNPs in the p53 tumor suppression network were investigated. Second, selection bias might be inevitable in this hospital-based case and control study. Third, although this was the largest association study for neuroblastoma susceptibility in Chinese children, the sample size was still moderate, especially for stratification analysis and inferred genotype analysis. Finally, our findings should be interpreted with caution since only Chinese Han population was recruited.

In conclusion, we validated the association of TP53 Arg72Pro and miR-34b/c rs4938723 polymorphisms with neuroblastoma susceptibility in Chinese children with a multi-center case-control study. These two SNPs may confer decreased neuroblastoma susceptibility cumulatively.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2019.06.008.

Conflict of Interest
None.

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References
[1] Stiller CA, and Parkin DM (1992). International variations in the incidence of neuroblastoma. *Int J Cancer* **52**, 538–543.
[2] Matthay K, Maris J, Schleiermacher G, Nakagawa A, Mackall C, Diller L, and Weiss W (2016). Neuroblastoma. *Nat Rev Dis Primers* **2**, 16078.
[3] Tolbert V, Coggins G, and Maris J (2017). Genetic susceptibility to neuroblastoma. *Curr Opin Genet Dev* **42**, 81–90.
[4] Ritenour L, Randall M, Bosse K, and Diskin S (2018). Genetic susceptibility to neuroblastoma: current knowledge and future directions. *Cell Tissue Res* **372**, 287–307.
[5] Capasso M, Devoto M, Hou C, Agha瞥 Zeh, S, Glesser JT, Attiyeh EF, Mosse YP, Kim C, Diskin SJ, and Cole KA, et al (2009). Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. *Nat Genet* **41**, 718–725.
[6] Diskin SJ, Capasso M, Schnepf RW, Cole KA, Attiyeh EF, Hou C, Diamond M, Carpenter EL, Winter C, and Lee H, et al (2012). Common variation at 6q16 within HACE1 and LIN28B influences susceptibility to neuroblastoma. *Nat Genet* **44**, 1126–1130.
[7] McDaniel LD, Conkrite KL, Chang X, Capasso M, Vaksman Z, Oldridge DA, Zachariou A, Horn M, Diamond M, and Hou C, et al (2017). Common variants upstream of MLF1 at 3q25 and within CPZ at 4p16 associated with neuroblastoma. *PLoS Genet* **13**(e1006787).
[8] Nguyen le B, Diskin SJ, Capasso M, Wang K, Diamond MA, Glesser J, Kim C, Attiyeh EF, Mosse YP, and Cole K, et al (2011). Phenotype restricted genome-wide association study using a gene-centric approach identifies three low-risk neuroblastoma susceptibility loci. *PLoS Genet* **7**(e1002026).
[9] Wang K, Diskin SJ, Zhang H, Attiyeh EF, Winter C, Hou C, Schnepf RW, Diamond M, Bosse K, and Meyes PA, et al (2011). Integrative genomics identifies LMO1 as a neuroblastoma oncogene. *Nature* **469**, 216–220.
[10] Capasso M, Diskin S, Cimmino F, Acieno G, Totaro F, Petrosino G, Pezone L, Diamond M, McDaniel L, and Hakonarson H, et al (2014). Common genetic variants in NEFL influence gene expression and neuroblastoma risk. *Cancer Res* **74**, 6913–6924.
[11] Capasso M, McDaniel LD, Cimmino F, Cirino A, Formicola D, Russell MR, Raman P, Cole KA, and Diskin SJ (2017). The functional variant rs43330 of CDKN1B is associated with risk of neuroblastoma. *J Cell Mol Med* **21**, 3224–3230.
[12] Capasso M, Diskin SJ, Totaro F, Longo L, De Mariano M, Russo R, Cimmino F, Hakonarson H, Tonini GP, and Devoto M, et al (2013). Replication of GWAS-identified neuroblastoma risk loci strengthens the role of BARD1 and affirms the cumulative effect of genetic variations on disease susceptibility. *Carcinogenesis* **34**, 605–611.
[13] Cimmino F, Avitabile M, Diskin SJ, Vaksman Z, Pignataro P, Formicola D, Cardinale A, Testori A, Koster J, and 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.
[14] Levine AJ, Hu W, and Fenz Z (2006). The P53 pathway: what questions remain to be explored? *Cell Death Differ* **13**, 1027–1036.
[15] Corney DC, Fleksen-Nikitin A, Godwin AK, Wang W, and Nikitin AY (2007). MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. *Cancer Res* **67**, 8433–8438.
[16] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, and Ridzon D, et al (2007). A microRNA component of the p53 tumour suppressor network. *Nature* **447**, 1130–1134.
[17] Matlashewski GJ, Tuck S, Pim D, Lamb P, Schneider J, and Crawford LV (1987). Primary structure polymorphism at amino acid residue 72 of human p53. *Mol Cell Biol* **7**, 961–963.
[18] Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovan F, Breuer J, Leigh IM, Matlashewski G, and Banks L (1998). Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* **393**, 229–234.
[19] Thomas M, Kalita A, Labrecque S, Pim D, Banks L, and Matlashewski G (1999). Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol* **19**, 1092–1100.
[20] Hashemi M, Mozafari-Roodi A, Bahari G, Talebi M, and Ghavami S (2019). Association between miR-34b/c rs4938723 polymorphism and risk of cancer: An updated meta-analysis of 27 case-control studies. *J Cell Biochem* **120**, 3306–3314.
[21] Li L, Wu J, Sima X, Bai P, Deng W, Deng X, Zhang L, and Gao L (2013). Interactions of miR-34b/c and TP-53 polymorphisms on the risk of nasopharyngeal carcinoma. *Tumour Biol* **34**, 1919–1923.
[22] Li L, Sima X, Bai P, Zhang L, Sun H, Liang W, Liu J, and Gao L (2012). Interactions of miR-34b/c and TP-53 polymorphisms on the risk of intracranial aneurysm. *Clin Dev Immunol* **2012**, 567586.
[23] Xu Y, Liu L, Liu J, Zhang Y, Zhu J, Chen J, Liu S, Liu Z, Shl H, and Shen H, et al (2011). A potentially functional polymorphism in the promoter region of miR-34b/c is associated with an increased risk for primary hepatocellular carcinoma. *Int J Cancer* **128**, 412–417.
[24] Oh J, Kim JW, Lee BE, Jang MJ, Chong SY, Park PW, Hwang SG, Oh D, and Kim NK (2014). Polymorphisms of the pri-miR-34b/c promoter and TP53 codon 72 are associated with risk of colon cancer. *Oncol Rep* **31**, 995–1002.
[25] Yuan F, Sun R, Chen P, Liang Y, Ni S, Quan Y, Huang J, Zhang L, and Gao L (2016). Combined analysis of pri-miR-34b/c rs4938723 and TP53 Arg72Pro with cervical cancer risk. *Tumour Biol* **37**, 6267–6273.
[26] Chen H, Sun Y, Pu Y, Bai P, Yuan F, Liang Y, Zhu B, Wang Y, Sun Y, and Zhu J, et al (2015). Pri-Mir-34b/C and TP-53 polymorphisms are associated with the susceptibility of papillary thyroid carcinoma: A case-control study. *Medicine (Baltimore)* **94**(e153).
[27] He J, Zhang X, Zhang J, Zhang R, Yang T, Zhu J, Xia H, and Zou Y (2018). LMO1 super-enhancer polymorphism rs2168101 G>T correlates with decreased neuroblastoma risk in Chinese children. *J Cancer* **9**, 1592–1597.
[28] He J, Zou Y, Liu X, Zhu J, Zhang J, Zhang R, Yang T, and Xia H (2018). Association of common genetic variants in pre-microRNAs and neuroblastoma susceptibility: A two-center study in Chinese children. *Mol Ther Nucleic Acids* **11**, 1–8.
[29] Cheng J, Zhao Z, Xin Y, Zhao P, Yang W, Zhou H, Zhang J, Gao Y, He J, and Li P (2018). Relevance of XPD polymorphisms to neuroblastoma risk in Chinese children: a four-center case-control study. *Aging (Albany NY)* **10**, 1989–2000.

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[30] Zhou H, Zhao Z, Chen S, Zhao J, Mo Y, Zhang J, He J, and Ruan J (2018). Polymorphisms in MYCN gene and neuroblastoma risk in Chinese children: a 3-center case-control study. Cancer Manag Res 10, 1807–1816.

[31] Zhang J, Zhuo Z, Li W, Zhu J, He J, and Su J (2018). XRCC1 gene polymorphisms and risk of neuroblastoma in Chinese children. Aging (Albany NY) 10, 2944–2953.

[32] Zhang J, Yang Y, Li W, Yan L, Zhang D, He J, and Wang J (2019). TP53 gene rs1042522 allele G decreases neuroblastoma risk: a two-centre case-control study. J Cancer 10, 467–471.

[33] Wang YZ, Zhuo ZJ, Fang Y, Li L, Zhang J, He J, and Wu XM (2018). Functional polymorphisms in hOGG1 gene and neuroblastoma risk in Chinese children. J Cancer 9, 4521–4526.

[34] He J, Wang F, Zhu J, Zhang Z, Zou Y, Zhang R, Yang T, and Xia H (2017). The TP53 gene rs1042522 C2G polymorphism and neuroblastoma risk in Chinese children. Aging (Albany NY) 9, 852–859.

[35] Zhu J, Wang M, Zhu M, He J, Wang JC, Jin L, Wang XF, Xiang QJ, and Wei Q (2015). Associations of PI3K-R1 and mTOR polymorphisms with esophageal squamous cell carcinoma risk and gene-environment interactions in Eastern Chinese populations. Sci Rep 5, 8250.

[36] Zhang J, Huang X, Xiao J, Yang Y, Zhou Y, Wang X, Liu Q, Yang J, Wang M, Tong N, Chu H, Wang M, Xue Y, Du M, Lu L, Zhang H, Wang F, Fang Y, and Qiu L, et al (2014). Pri-miR-124 rs531564 and pri-miR-34b/c rs4938723 polymorphisms are associated with decreased risk of esophageal squamous cell carcinoma in Chinese populations. PLoS One 9:e100055.

[37] Zhang J, Huang X, Xiao J, Yang Y, Zhou Y, Wang X, Liu Q, Yang J, Wang M, and Qiu L, et al (2014). Pri-miR-124 rs531564 and pri-miR-34b/c rs4938723 polymorphisms are associated with decreased risk of esophageal squamous cell carcinoma in Chinese populations. PLoS One 9:e100055.

[38] Hashemi M, Bahari G, Naderi M, Sadeghi-Bojd S, and Taheri M (2016). Pri-miR-34b/c rs938723 polymorphism is associated with the risk of childhood acute lymphoblastic leukemia. Cancer Genet 209, 493–496.

[39] Liu CJ, Ma XW, Zhang XJ, and Shen SQ (2017). pri-miR-34b/c rs4938723 polymorphism is associated with hepatocellular carcinoma risk: a case-control study in a Chinese population. Int J Mol Epidemiol Genet 8, 1–7.

[40] Pan XM, Sun RF, Li ZH, Guo XM, Qin HJ, and Gao LB (2015). Pri-miR-34b/c rs4938723 polymorphism is associated with a decreased risk of gastric cancer. Genet Test Mol Biomarkers 19, 198–202.

[41] Hashemi M, Danesh H, Bzihani F, Narouie B, Sotoudeh M, Nourizadeh A, Shariffaghda F, Bahari G, and Taheri M (2017). Pri-miR-34b/c rs4938723 polymorphism increases the risk of prostate cancer. Neoplasia 19, 1441–1446.

[42] Tajerian T, Lee JY, and Lee YS (2018). miRNA-34d suppresses EMT in an in vitro model of breast cancer. Cancer Cell 23, 271–285.

[43] Zhang J, Yang Y, Li W, Yan L, Zhang D, He J, and Wang J (2019). TP53 gene rs1042522 allele G decreases neuroblastoma risk: a two-centre case-control study. J Cancer 10, 467–471.

[44] Wei JS, Song YK, Durinck S, Chen QR, Cheuk AT, Tsang P, Zhang Q, Thielle CJ, Slack A, and Sholet J, et al (2008). The MYCN oncogene is a direct target of miR-34a. Oncogene 27, 5204–5213.

[45] Diskin SJ, Capasso M, Diamond M, Oldridge DA, Conkrite K, Bosse KR, Russell MR, Iolascon A, Hakonarson H, and Devoto M, et al (2014). Rare variants in TP53 and susceptibility to neuroblastoma. J Natl Cancer Inst 106, dju047.

[46] Cattelan S, Ferrari-Amorotti G, Galavotti S, Defferrari R, Tanno B, Cialfi S, Vergalli J, Fragliasso V, Guerzoni C, and Manzotti G, et al (2012). The p53 codon 72 Pro/Pro genotype identifies poor-prognosis neuroblastoma patients: correlation with reduced apoptosis and enhanced senescence by the p53-72P isoform. Neoplasia 14, 634–643.

[47] Zhang J, Huang X, Xiao J, Yang Y, Zhou Y, Wang X, Liu Q, Yang J, Wang M, and Qiu L, et al (2014). Pri-miR-124 rs531564 and pri-miR-34b/c rs4938723 polymorphisms are associated with decreased risk of esophageal squamous cell carcinoma in Chinese populations. PLoS One 9:e100055.

[48] Yi DH, Wang BG, Zhang XP, Liu H, and Liu YF (2014). Pri-miR-34b/c rs4938723 TC heterozygote is associated with increased cancer risks: evidence from published data. Tumour Biol 35, 11967–11975.

[49] Hashemi M, Bahari G, Naderi M, Sadeghi-Bojd S, and Taheri M (2016). Pri-miR-34b/c rs938723 polymorphism is associated with the risk of childhood acute lymphoblastic leukemia. Cancer Genet 209, 493–496.

[50] Liu CJ, Ma XW, Zhang XJ, and Shen SQ (2017). pri-miR-34b/c rs4938723 polymorphism is associated with hepatocellular carcinoma risk: a case-control study in a Chinese population. Int J Mol Epidemiol Genet 8, 1–7.

[51] Pan XM, Sun RF, Li ZH, Guo XM, Qin HJ, and Gao LB (2015). Pri-miR-34b/c rs4938723 polymorphism is associated with a decreased risk of gastric cancer. Genet Test Mol Biomarkers 19, 198–202.

[52] Hashemi M, Danesh H, Bzihani F, Narouie B, Sotoudeh M, Nourizadeh A, Shariffaghda F, Bahari G, and Taheri M (2017). Pri-miR-34b/c rs4938723 polymorphism increases the risk of prostate cancer. Neoplasia 19, 1441–1446.

[53] Tajerian T, Lee JY, and Lee YS (2018). miRNA-34d suppresses EMT in an in vitro model of breast cancer. Cancer Cell 23, 271–285.

[54] Wei JS, Song YK, Durinck S, Chen QR, Cheuk AT, Tsang P, Zhang Q, Thielle CJ, Slack A, and Sholet J, et al (2008). The MYCN oncogene is a direct target of miR-34a. Oncogene 27, 5204–5213.

[55] Diskin SJ, Capasso M, Diamond M, Oldridge DA, Conkrite K, Bosse KR, Russell MR, Iolascon A, Hakonarson H, and Devoto M, et al (2014). Rare variants in TP53 and susceptibility to neuroblastoma. J Natl Cancer Inst 106, dju047.