Translational Oncology
www.transonc.com

Volume 12 Number 10 October 2019 pp. 1282–1288 1282

Association of *miR-34b/c* rs4938723 and *TP53* Arg72Pro Polymorphisms with Neuroblastoma Susceptibility: Evidence from Seven Centers[1]

Le Li[*,2], Jinhong Zhu[*,†,2], Tongyi Lu[*,2], Wei Liu[*], Jue Tang[*], Jiao Zhang[‡], Yizhen Wang[§], Yong Li[¶], Suhong Li[#], Haixia Zhou[**], Huimin Xia[*], Jing He[*] and Jiwen Cheng[††]

[*]Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510623, Guangdong, China; [†]Department of Clinical Laboratory, Biobank, Harbin Medical University Cancer Hospital, Harbin 150040, Heilongjiang, China; [‡]Department of Pediatric Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China; [§]Department of Pathology, Anhui Provincial Children's Hospital, Hefei 230051, Anhui, China; [¶]Department of Pediatric Surgery, Hunan Children's Hospital, Changsha 410004, Hunan, China; [#]Department of Pathology, Children Hospital and Women Health Center of Shanxi, Taiyuan 030013, Shannxi, China; [**]Department of Hematology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, Zhejiang, China; [††]Department of Pediatric Surgery, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, Shaanxi, China

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%

Address all correspondence to: Jing He, Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 9 Jinsui Road, Guangzhou 510623, Guangdong, China. or Jiwen Cheng, Department of Pediatric Surgery, the Second Affiliated Hospital of Xi'an Jiaotong University, No. 157 West 5 Road, Xi'an 710004, Shaanxi, China. E-mail: hejing198374@gmail.com, chengjiwen68@163.com

[1]Novelty: In this seven-center case-control study with 819 cases and 1780 controls, we assessed the association of the *TP53* Arg72Pro and *miR-34b/c* rs4938723 polymorphisms with neuroblastoma susceptibility for Chinese children. We found both of these two polymorphisms were associated with significantly deceased neuroblastoma susceptibility. Moreover, cumulative effects of the two polymorphisms were observed, evidenced by more significantly decreased neuroblastoma risk in subjects with variant alleles of both polymorphisms than in those with either one alone. Significant findings were confirmed by false positive report possibility analysis.

[2]These authors contribute equally.

Received 20 May 2019; Revised 26 June 2019; Accepted 28 June 2019

© 2019 The Authors. Published by Elsevier Ltd. on behalf of Neoplasia Press, Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1936-5233/19
https://doi.org/10.1016/j.tranon.2019.06.008

CI = 0.37-0.69; CG/TT vs. CC/TT: adjusted OR = 0.71, 95% CI = 0.55-0.92), suggesting cumulative effects of the polymorphisms. False positive report possibility analysis further verified that our findings are noteworthy. Overall, we confirmed that *miR-34b/c* rs4938723 and *TP53* Arg72Pro conferred decreased neuroblastoma risk and two polymorphisms exerted stronger protective effects against neuroblastoma than either one alone.

Translational Oncology (2019) 12, 1282–1288

Introduction

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 (GWASs) have identified a number of neuroblastoma susceptibility loci in the *CASC15*, *BARD1*, *DUSP12*, *DDX4*, *IL31RA*, *HSD17B12*, *LMO1*, *HACE1*, *LIN28B*, *MLF1*, and *CPZ* 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 *NEFL*, *CDKN1B* and *BARD1* genes [10–13].

Tumor suppressing protein p53 is a transcription factor. It suppresses tumorigenesis by orchestrating the transcriptional activation of multiple target genes to fight against DNA damage, cellular stress, and excessive mitogenic stimulation [14]. Numerous genes are involved in p53 tumor suppressor network, including *p21*, *cyclin G*, *MDM-2*, *GADD-45*, *PTEN*, *and TSC-2*. Moreover, p53 network was further complicated by the finding that p53 can execute tumor suppressing function by transcriptionally regulating microRNAs (miRNAs), especially *miR-34* family [15,16]. *miR-34* family consists of three mature miRNAs, *miR-34a*, *miR-34b*, and *miR-34c*. *miR-34* family encoding genes are direct targets of p53 in the transcriptional level, with evolutionarily conserved p53 binding sites located upstream the miRNA encoding sequence [15,16].

Genetic variations may change the expression levels and structures of tumor repressor genes and alter tumor repressing function. A genetic polymorphism Arg72Pro at codon 72 (rs1042522 C > G) of p53 protein can affect protein function biochemically and biologically [17–19]. Moreover, a functional common single nucleotide polymorphism (SNP) rs4938723 T > C was identified in the promoter region of the *pri-miR-34b/c* encoding gene [20]. This SNP is located in a typical CpG island, 423-bp upstream from the transcription start site. These two SNPs have been broadly investigated for their association with cancer susceptibility.

Due to the close relationship between p53 and *miR-34b/c*, a number of studies were launched to investigate the risk effects of *TP53* rs1042522 C > G and *miR-34b/c* rs4938723 T > C polymorphisms jointly on the different types of cancer, including primary hepatocellular carcinoma, intracranial aneurysm, nasopharyngeal carcinoma, colorectal cancer, cervical cancer, and papillary thyroid carcinoma [21–26]. However, whether they jointly confer susceptibility to neuroblastoma needed to be explored in a large well-designed case control study. Therefore, we investigated the association of these two SNPs 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 72controls) [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 Second 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.

Table 1. Associations Between *TP53* and *miR-34b/c* Polymorphisms and Neuroblastoma Susceptibility

Genotype	Cases (N = 819)	Controls (N = 1780)	$P^{\dagger}$	Crude OR (95% CI)	P	Adjusted OR (95% CI) [‡]	$P^{\ddagger}$
TP53 rs1042522 C > G (HWE = 0.541) [§]							
CC	285 (34.80)	544 (30.58)		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			.072	0.92 (0.82−1.04)	.164	0.92 (0.82−1.03)	.156
Dominant	534 (65.20)	1235 (69.42)	.032	**0.83 (0.69−0.98)**	**.032**	**0.82 (0.69−0.98)**	**.031**
Recessive	660 (80.59)	1435 (80.66)	.963	1.01 (0.82−1.24)	.963	1.00 (0.81−1.24)	.988
C	945 (57.69)	1979 (55.62)		1.00		1.00	
G	693 (42.31)	1579 (44.38)	.162	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	
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)	.564	1.08 (0.83−1.41)	.578
Additive			<.0001	**0.84 (0.74−0.95)**	**.006**	**0.84 (0.74−0.95)**	**.006**
Dominant	348 (43.34)	970 (54.56)	<.0001	**0.64 (0.54−0.75)**	**<.0001**	**0.64 (0.54−0.75)**	**<.0001**
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	
C	454 (28.27)	1144 (32.17)	.005	**0.83 (0.73−0.95)**	**.005**	**0.83 (0.73−0.95)**	**.005**

OR, odds ratio; CI, confidence interval.
[†] χ^2 test for genotype distributions between neuroblastoma cases and cancer-free controls.
[‡] Adjusted for age and gender.
[§] There were missing values for genotyping that failed.

Statistical Analysis

Frequency distributions of demographic variables and genotype were compared between cases and controls using χ^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 different genetic models, I) homozygous (VV vs. WW), II) heterozygous (WV vs. WW), III) dominant (WV/VV vs. WW), IV) recessive (VV vs. WW/WV), and allele contrast (V vs. W), with W and V indicating wild-type and variant allele of a SNP, respectively. Multivariate analysis was conducted using unconditional logistic regression, with adjustment for age and gender. To dissect out real significant associations from chance findings, we performed the false positive report possibility (FPRP) analysis for the significant 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.

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

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

Variables	rs1042522 (cases/controls)		AOR (95% CI) [†]	$P^{\dagger}$	rs4938723 (cases/controls)		AOR (95% CI) [†]	$P^{\dagger}$
	CC	CG/GG			TT	TC/CC		
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	299/709	**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)	.161	160/808	97/970	**0.51 (0.39−0.66)**	**<.0001**
Retroperitoneal	93/544	188/1235	0.88 (0.68−1.16)	.369	154/808	117/970	**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+ 4 s	154/544	288/1235	0.83 (0.66−1.03)	.089	238/808	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.
[†] Adjusted for age and gender, omitting the corresponding stratify factor.

Table 3. Inferred Genotypes of *miR-34b/c* and *TP53* Gene and Their Association with the Neuroblastoma Susceptibility

Genotypes		Cases	Controls	OR (95% CI)	*P*	AOR (95% CI)‡	*P*‡
rs1042522	rs4938723	(n = 803)†	(n = 1777)†				
CC	TT	163 (20.30)	246 (13.84)	1.00		1.00	
CC	TC	85 (10.59)	236 (13.28)	**0.50 (0.37−0.69)**	**<.0001**	**0.51 (0.37−0.69)**	**<.0001**
CC	CC	33 (4.11)	61 (3.43)	0.76 (0.48−1.20)	.237	0.76 (0.48−1.21)	.246
CG	TT	206 (25.65)	407 (22.90)	**0.71 (0.55−0.91)**	**.008**	**0.71 (0.55−0.92)**	**.008**
CG	TC	109 (13.57)	405 (22.79)	**0.38 (0.28−0.50)**	**<.0001**	**0.38 (0.28−0.50)**	**<.0001**
CG	CC	50 (6.23)	78 (4.39)	0.90 (0.60−1.34)	.591	0.89 (0.60−1.34)	.588
GG	TT	86 (10.71)	154 (8.67)	0.78 (0.56−1.08)	.135	0.78 (0.56−1.08)	.139
GG	TC	48 (5.98)	155 (8.72)	**0.43 (0.30−0.63)**	**<.0001**	**0.43 (0.30−0.63)**	**<.0001**
GG	CC	23 (2.86)	35 (1.97)	0.92 (0.52−1.61)	.764	0.91 (0.52−1.60)	.750

OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval.

† There were missing value for genotyping failed.

‡ Obtained in logistic regression models with adjustment for age and gender.

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

Table 4. False-Positive Report Probability Analysis for Significant Findings

Genotype	OR (95% CI)	*P*†	Statistical power‡	Prior probability				
				0.25	0.1	0.01	0.001	0.0001
rs1042522 C > G								
CG vs. CC	0.80 (0.67−0.97)	.022	0.992	**0.062**	**0.166**	0.687	0.957	0.996
CG/GG vs. CC	0.83 (0.69−0.98)	.032	0.991	**0.089**	0.226	0.763	0.970	0.997
>18	0.78 (0.62−0.98)	.032	0.910	**0.095**	0.240	0.777	0.972	0.997
Males	0.78 (0.62−0.99)	.041	0.911	**0.119**	0.288	0.817	0.978	0.998
Mediastinum	0.68 (0.50−0.91)	.011	0.532	**0.056**	**0.152**	0.663	0.952	0.995
rs4938723 T > C								
TC vs. TT	0.54 (0.45−0.65)	<.0001	0.021	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
TC/CC vs. TT	0.64 (0.54−0.75)	<.0001	0.291	**0.000**	**0.000**	**0.000**	**0.001**	**0.005**
CC vs. TT/CT	1.40 (1.08−1.81)	.010	0.708	**0.041**	**0.114**	0.585	0.934	0.993
C vs. T	0.83 (0.73−0.95)	.005	0.999	**0.015**	**0.043**	0.331	0.833	0.980
TC/CC vs. TT								
≤18	0.63 (0.49−0.82)	.0007	0.342	**0.006**	**0.018**	**0.168**	0.671	0.953
>18	0.64 (0.52−0.80)	<.0001	0.352	**0.001**	**0.002**	**0.017**	**0.151**	0.641
Females	0.54 (0.42−0.70)	<.0001	0.060	**0.000**	**0.000**	**0.005**	**0.052**	0.353
Males	0.72 (0.58−0.90)	.004	0.734	**0.015**	**0.043**	**0.333**	0.834	0.981
Adrenal gland	0.51 (0.39−0.66)	<.0001	0.024	**0.000**	**0.000**	**0.003**	**0.027**	0.217
Retroperitoneal	0.63 (0.49−0.82)	.0005	0.339	**0.004**	**0.013**	**0.127**	0.596	0.937
I + II+ 4 s	0.70 (0.57−0.86)	.0009	0.666	**0.004**	**0.012**	**0.118**	0.575	0.931
III + IV	0.57 (0.45−0.72)	<.0001	0.089	**0.000**	**0.000**	**0.002**	**0.024**	0.201
Genotypes§								
CC/TC vs. CC/TT	0.50 (0.37−0.69)	<.0001	0.065	**0.001**	**0.002**	**0.025**	0.208	0.725
CG/TT vs. CC/TT	0.71 (0.55−0.91)	.008	0.847	**0.026**	**0.075**	0.471	0.900	0.989
CG/TC vs. CC/TT	0.38 (0.28−0.50)	<.0001	0.001	**0.000**	**0.000**	0.000	0.000	0.000
GG/TC vs. CC/TT	0.43 (0.30−0.63)	<.0001	0.016	**0.002**	**0.007**	**0.071**	0.434	0.885

OR, odds ratio; CI, confidence interval.

[a] χ^2 Test was used to calculate the genotype frequency distributions.

‡ Statistical power was calculated using the number of observations in each subgroup and the corresponding ORs and *P* values in this table.

§ The genotypes were constructed in the order of rs1042522 and rs4938723.

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 *miR-34a* and *miR-34b/c* genes are mapped to Chr.1p36 and Chr.11q23 [42]. Mechanistic study revealed that *miR-34b*/*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 nueroblastoma. 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 explore 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 rs78378222 polymorphisms and susceptibility to neuroblastoma [45]. However, Cattelani et al. found lack of association between minor allele of *TP53* rs1042522 and neuroblastoma 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 susceptibility 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 polymorphism 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 susceptibility 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 polymorphism 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.

Acknowledgements

This work was supported by grants from the Pearl River S&T Nova Program of Guangzhou (No: 201710010086). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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, Nakagawara 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, Asgharzadeh S, Glessner 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**, 718–723.
[6] Diskin SJ, Capasso M, Schnepp 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, Glessner 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, Schnepp RW, Diamond M, Bosse K, and Mayes PA, et al (2011). Integrative genomics identifies LMO1 as a neuroblastoma oncogene. *Nature* **469**, 216–220.
[10] Capasso M, Diskin S, Cimmino F, Acierno 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 rs34330 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 Feng Z (2006). The P53 pathway: what questions remain to be explored? *Cell Death Differ* **13**, 1027–1036.
[15] Corney DC, Flesken-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, Mantovani 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, Moazeni-Roodi A, Bahari G, Taheri 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 TP53 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, Shi 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 colorectal 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 P, Sun R, Pu Y, Bai P, Yuan F, Liang Y, Zhou 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**:e1536.
[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, Zhuo 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.

[30] Zhou H, Zhuo 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 C>G 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 JQ, and Wei Q (2015). Associations of PI3KR1 and mTOR polymorphisms with esophageal squamous cell carcinoma risk and gene-environment interactions in Eastern Chinese populations. *Sci Rep* **5**, 8250.
[36] Zhu J, Wang M, He J, Zhu M, Wang JC, Jin L, Wang XF, Yang YJ, Xiang JQ, and Wei Q (2016). Polymorphisms in the AKT1 and AKT2 genes and oesophageal squamous cell carcinoma risk in an Eastern Chinese population. *J Cell Mol Med* **20**, 666–677.
[37] Chang J, Tian J, Yang Y, Zhong R, Li J, Zhai K, Ke J, Lou J, Chen W, and Zhu B, et al (2018). A rare missense variant in TCF7L2 associates with colorectal cancer risk by interacting with a GWAS-identified regulatory variant in the MYC enhancer. *Cancer Res* **78**, 5164–5172.
[38] Chang J, Tian J, Zhu Y, Zhong R, Zhai K, Li J, Ke J, Han Q, Lou J, and Chen W, et al (2018). Exome-wide analysis identifies three low-frequency missense variants associated with pancreatic cancer risk in Chinese populations. *Nat Commun* **9**, 3688.
[39] Chang J, Zhong R, Tian J, Li J, Zhai K, Ke J, Lou J, Chen W, Zhu B, and Shen N, et al (2018). Exome-wide analyses identify low-frequency variant in CYP26B1 and additional coding variants associated with esophageal squamous cell carcinoma. *Nat Genet* **50**, 338–343.
[40] 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.
[41] Hermeking H (2007). p53 enters the microRNA world. *Cancer Cell* **12**, 414–418.
[42] Hermeking H (2010). The miR-34 family in cancer and apoptosis. *Cell Death Differ* **17**, 193–199.
[43] Yu Z, Kim J, He L, Creighton CJ, Gunaratne PH, Hawkins SM, and Matzuk MM (2014). Functional analysis of miR-34c as a putative tumor suppressor in high-grade serous ovarian cancer. *Biol Reprod* **91**, 113.
[44] Wei JS, Song YK, Durinck S, Chen QR, Cheuk AT, Tsang P, Zhang Q, Thiele CJ, Slack A, and Shohet 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] Cattelani 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, Zhong 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 rs4938723 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, Bizhani F, Narouie B, Sotoudeh M, Nouralizadeh A, Sharifiaghdas F, Bahari G, and Taheri M (2017). Pri-miR-34b/c rs4938723 polymorphism increased the risk of prostate cancer. *Cancer Biomark* **18**, 155–159.
[53] Tong N, Chu H, Wang M, Xue Y, Du M, Lu L, Zhang H, Wang F, Fang Y, and Li J, et al (2016). Pri-miR-34b/c rs4938723 polymorphism contributes to acute lymphoblastic leukemia susceptibility in Chinese children. *Leuk Lymphoma* **57**, 1436–1441.
[54] Dumont P, Leu JI, Della Pietra 3rd AC, George DL, and Murphy M (2003). The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* **33**, 357–365.
[55] Xu B, Zhu Y, Tang Y, Zhang Z, and Wen Q (2018). Rs4938723 polymorphism is associated with susceptibility to hepatocellular carcinoma risk and is a protective factor in leukemia, colorectal, and esophageal cancer. *Med Sci Monit* **24**, 7057–7071.