


RESEARCH ARTICLE

MYCN gene polymorphisms and Wilms tumor susceptibility in Chinese children

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Funding information

Zhejiang Provincial Science and Technology Animal Experimental Platform Project, Grant/Award Number: 016C37113; Scientific Research Foundation of Wenzhou, Grant/Award Number: 2015Y0492; Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease, Grant/Award Number: 2019B030301004; Pearl River S&T Nova Program of Guangzhou, Grant/Award Number: 201710010086

Abstract

Background: Wilms tumor, derived from embryonic cells, accounts for a large proportion of pediatric renal tumors. MYCN encoded by MYCN proto-oncogene, a member of the MYC family, is a BHLH transcription factor. It plays a critical role in tumorigenesis and predicts poor clinical outcomes in various types of cancer. However, the role of MYCN remained unclarified in Wilms tumor. In this study, we investigated the association between MYCN gene polymorphisms and Wilms tumor susceptibility.

Methods: Four MYCN gene polymorphisms (rs57961569 G > A, rs9653226 T > C, rs13034994 A > G, and rs60226897 G > A) were genotyped in 183 cases and 603 controls. Adjusted odds ratios (AORs) and 95% confidence intervals (CIs) were calculated to evaluate the association between MYCN gene polymorphisms and Wilms tumor susceptibility.

Results: Overall, no significant association was found for any of the four MYCN gene polymorphisms. Interestingly, in the stratification analysis, the rs57961569 was found to be associated with decreased Wilms tumor susceptibility in the children older than 18 months (AOR = 0.65, 95% CI = 0.42-1.00, $P = .050$). Moreover, older children carrying 2-4 risk genotypes were at increased risk of Wilms tumor (OR = 1.55, 95% CI = 1.001-2.40, $P = .0497$). Haplotype GCAA was shown to significantly increased Wilms tumor risk (AOR = 2.40, 95% CI = 1.12-5.14, $P = .024$).

Conclusion: Our study demonstrated that these MYCN gene polymorphisms might be low penetrant variants in Wilms tumor.

KEYWORDS

MYCN, polymorphism, susceptibility, Wilms tumor

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1 | INTRODUCTION

Wilms tumor is a classic embryonal tumor in the developing kidney, with a 90% cure rate.¹⁻³ Despite the high cure rate in patients with low-risk Wilms tumor, patients with poor histologic and molecular characteristics, bilateral lesions, and recurrent disease have a much lower survival rate and deserve more attentions.⁴ Moreover, approximately 24% of Wilms tumor survivors are at a higher risk of other diseases, including second primary cancers, infertility, and cardiac disease.^{5,6} In the clinical, although Wilms tumor patients have a high cure rate, high-risk patients still have a very poor prognosis. Therefore, we must fully understand the polymorphisms in Wilms

tumor patients to find more effective, cheaper, and safer treatment. At the same time, understanding the genetic inheritance of Wilms tumor is also an important premise to improve the prognosis of patients.

Some causal gene mutations have been reported in Wilms tumor. For instance, individuals with *WT1* mutations have an enormous risk of Wilms tumor.^{7,8} A study by Ciceri et al suggested that constitutional anomalies of *CHEK2* play an important role in the development of Wilms tumor.⁹ Moreover, a series of genetic alterations were identified associate with Wilms tumor susceptibility, including *WTX*,¹⁰ *CITED1*,¹¹ *SIX1*,¹² *SIX2*,¹³ *TP53*,^{14,15} *HACE1*,¹⁶ *LIN28B*,¹⁷ and *KRAS*.¹⁸ Nevertheless, these genetic variations are

TABLE 1 Association between *MYCN* gene polymorphisms and Wilms tumor susceptibility

Genotype	Cases (N = 183)	Controls (N = 603)	<i>P</i> ^a	Crude OR (95% CI)	<i>P</i>	Adjusted OR (95% CI) ^b	<i>p</i> ^b
rs57961569 G > A (HWE = 0.582)							
GG	90 (49.18)	259 (42.95)		1.00		1.00	
GA	74 (40.44)	277 (45.94)		0.77 (0.54-1.09)	.142	0.77 (0.54-1.09)	.140
AA	19 (10.38)	67 (11.11)		0.82 (0.47-1.43)	.479	0.82 (0.47-1.44)	.481
Additive			.325	0.85 (0.66-1.10)	.215	0.85 (0.66-1.10)	.215
Dominant	93 (50.82)	344 (57.05)	.138	0.78 (0.56-1.08)	.138	0.78 (0.56-1.08)	.137
Recessive	164 (89.62)	536 (88.89)	.782	0.93 (0.54-1.59)	.782	0.93 (0.54-1.59)	.787
rs9653226 T > C (HWE = 0.719)							
TT	59 (32.24)	201 (33.33)		1.00		1.00	
TC	89 (48.63)	298 (49.42)		1.02 (0.70-1.48)	.928	1.01 (0.70-1.48)	.944
CC	35 (19.13)	104 (17.25)		1.15 (0.71-1.85)	.577	1.15 (0.71-1.85)	.580
Additive			.840	1.06 (0.84-1.35)	.613	1.06 (0.84-1.35)	.618
Dominant	124 (67.76)	402 (66.67)	.783	1.05 (0.74-1.50)	.783	1.05 (0.74-1.49)	.796
Recessive	148 (80.87)	499 (82.75)	.560	1.14 (0.74-1.74)	.560	1.14 (0.74-1.74)	.556
rs13034994 A > G (HWE = 0.581)							
AA	104 (56.83)	350 (58.04)		1.00		1.00	
AG	67 (36.61)	222 (36.82)		1.02 (0.72-1.44)	.931	1.02 (0.72-1.45)	.916
GG	12 (6.56)	31 (5.14)		1.30 (0.65-2.63)	.460	1.30 (0.65-2.63)	.458
Additive			.759	1.08 (0.82-1.41)	.603	1.08 (0.82-1.42)	.592
Dominant	79 (43.17)	253 (41.96)	.771	1.05 (0.75-1.47)	.771	1.06 (0.75-1.48)	.756
Recessive	171 (93.44)	572 (94.86)	.461	1.30 (0.65-2.58)	.462	1.30 (0.65-2.58)	.462
rs60226897 G > A (HWE = 0.377)							
GG	92 (50.27)	293 (48.59)		1.00		1.00	
GA	69 (37.70)	248 (41.13)		0.89 (0.62-1.26)	.504	0.89 (0.62-1.27)	.508
AA	22 (12.02)	62 (10.28)		1.13 (0.66-1.94)	.657	1.14 (0.66-1.95)	.647
Additive			.642	1.00 (0.78-1.28)	.992	1.00 (0.78-1.28)	.981
Dominant	91 (49.73)	310 (51.41)	.690	0.94 (0.67-1.30)	.690	0.94 (0.67-1.30)	.697
Recessive	161 (87.98)	541 (89.72)	.505	1.19 (0.71-2.00)	.505	1.20 (0.71-2.01)	.496
Combined effect of risk genotypes							
0-1	85 (46.45)	328 (54.39)		1.00		1.00	
2-4	98 (53.55)	275 (45.61)	.059	1.38 (0.99-1.92)	0.058	1.38 (0.99-1.92)	.058

^a χ^2 test for genotype distributions between Wilms tumor patients and controls.

^bAdjusted for age and gender.

^cRisk genotype was with rs57961569 GG, rs9653226 TC/CC, rs13034994 AG/GG, and rs60226897 AA.

not sufficient to explain the whole genetic pathogenesis involved in Wilms tumor.

MYCN encoded by the MYCN proto-oncogene is a MYC gene family member. This protein plays an indispensable role in the processes of cell growth, proliferation, differentiation, and apoptosis.¹⁹⁻²¹ In recent years, several groups reported amplified MYCN gene in different human cancers, such as human neuroblastoma, small cell lung carcinomas, astrocytoma, and retinoblastoma.^{22,23} In addition, MYCN copy number increased in 17.5% of basal cell carcinoma patients.²⁴ Despite all these achievements, the implication of MYCN gene in Wilms tumor remains not fully clarified, especially the effects of potentially functional single nucleotide polymorphisms (SNPs). Thus, we conducted this study to identify the association between MYCN SNPs and Wilms tumor susceptibility.

2 | MATERIALS AND METHODS

2.1 | Study subjects

In this hospital-based epidemiological study, we recruited 183 patients and 603 controls from Yuying Children's Hospital of Wenzhou Medical University and Guangzhou Women and Children's Medical Center. Age, gender, and clinical stage distributions of all participants are summarized in Table S1. All the guardians of participants were voluntary to donate blood samples and signed informed consent forms. All individuals were independently recruited. This study was permitted by the Institutional Review Board of both the centers.

2.2 | SNP selection and genotyping

Four polymorphisms (rs57961569 G > A, rs9653226 T > C, rs13034994 A > G, and rs60226897 G > A) in the MYCN gene were selected in this study as we described previously.²⁵ Specific criteria of SNPs selection and genotyping methods have been described in our previous study.²⁶⁻²⁸ All the above four polymorphisms are located at the binding sites of transcription factors, which have potential effects on the binding ability of transcription factors. In addition, they all have a minor allele frequency greater than 5% in the Chinese population.

2.3 | Statistical analysis

Hardy-Weinberg equilibrium (HWE) among controls was calculated using goodness-of-fit chi-squared test. And the differences in demographic variables and SNPs frequencies between cases and controls were tested using two-sided chi-squared test. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were adopted to appraise the association of MYCN gene SNPs with Wilms tumor risk. Difference between groups was deemed to be effective significant when $P < .05$. All of the statistical analyses were processed with the version 9.4 SAS (SAS Institute, Cary, NC).

TABLE 2 Stratification analysis of risk genotypes with tumor susceptibility

Variables	rs57961569 (cases/ controls)		Risk genotypes (cases/ controls)		P ^a	AOR (95% CI) ^a	P	OR (95% CI)	AOR (95% CI) ^a	P ^a
	GG	GA/AA	0-1	2-4						
Age, month										
≤18	35/124	41/144	35/135	41/133	.937	1.02 (0.61-1.70)	.973	1.19 (0.71-1.98)	1.18 (0.71-1.96)	.534
>18	55/135	52/200	50/193	57/142	.050	0.65 (0.42-1.00)	.044	1.55 (1.001-2.40)	1.54 (0.99-2.38)	.056
Gender										
Female	40/119	41/148	37/142	44/125	.447	0.82 (0.50-1.36)	.447	1.35 (0.82-2.23)	1.35 (0.82-2.23)	.234
Male	50/140	52/196	48/186	54/150	.188	0.74 (0.48-1.16)	.190	1.40 (0.90-2.18)	1.40 (0.90-2.18)	.139
Clinical stages										
I + II	34/259	33/344	30/328	37/275	.248	0.74 (0.45-1.23)	.224	1.47 (0.89-2.44)	1.44 (0.86-2.39)	.163
III + IV	46/259	52/344	48/328	50/275	.456	0.85 (0.55-1.31)	.461	1.24 (0.81-1.91)	1.26 (0.82-1.93)	.298

^aAdjusted for age and gender, without the corresponding stratify factor. Bold values indicate the 95% CI excluded 1 or $P < .05$.

3 | RESULTS

3.1 | Characteristics of study population

The characteristics of subjects from Wenzhou and Guangzhou are summarized in Table S1. All the participants were under the age of 15 years, and the mean age was 29.64 months (± 25.71 , range = 1-144 months) for cases and 29.00 months (± 24.00 , range = 0.07-156 months) for controls, respectively. The discrepancy in age and gender between the cases and controls was not statistically significant ($P = .486$ for age and $P = .997$ for gender). In the case group, 6.56% of the patients were in clinical stage I, 30.05% were in II, 31.69% were in III, 21.86% were in IV, and 9.84% were not available (NA).

3.2 | Correlation of MYCN gene polymorphisms with Wilms tumor risk

The detailed results are presented in Table 1. None of the four selected polymorphisms showed obvious deviation from the HWE in the control groups ($P = .582$ for the rs57961569 G > A polymorphism, $P = .719$ for the rs9653226 T > C polymorphism, $P = .581$ for the rs13034994 A > G polymorphism, $P = .377$ for the rs60226897 G > A polymorphism). Overall, no significant association was found between MYCN gene polymorphisms and Wilms tumor risk. No significant results were observed for the combined effect of risk genotypes either.

3.3 | Stratification analysis

Stratification analysis was performed according to age, sex, and clinical stages (Table 2). The rs57961569 GA/AA genotypes significantly

decreased Wilms tumor susceptibility in the children older than 18 months (adjusted OR = 0.65, 95% CI = 0.42-1.00, $P = .050$). In addition, children with 2-4 risk genotypes have a greater risk of developing Wilms tumor than those with 0-1 risk genotype (OR = 1.55, 95% CI = 1.001-2.40, $P = .0497$). However, the association was not statistically significant after adjusting for age and gender, which turns out that age and gender were confounding factors.

3.4 | Haplotype analysis

As shown in the Table 3, it is obvious that haplotype GCAA significantly increased Wilms tumor risk (adjusted OR = 2.40, 95% CI = 1.12-5.14, $P = .024$) compared to reference haplotype GTAG.

4 | DISCUSSION

We performed the current study to investigate the association of MYCN gene polymorphisms with Wilms tumor risk. We found no significant association between four included polymorphisms of MYCN and Wilms tumor risk in 183 cases and 603 controls. However, stratification and haplotype analyses revealed the potential contribution of MYCN polymorphisms to Wilms tumor risk.

Several groups have explored the influence of MYCN gene on Wilms tumor. It is proved that the expression level of MYCN increased in Wilms tumor,²⁹⁻³² and MYCN gene can up-regulate the expression of CRABP-II in Wilms tumor patients to promote tumor progression.³³ A study executed by Williams et al showed that MYCN gene contributed to the development of Wilms tumor through manifold mechanisms.³⁴ In addition, the effect of MYCN gene polymorphisms

TABLE 3 The frequency of inferred haplotypes of MYCN gene based on observed genotypes and their association with the risk of Wilms tumor

Haplotypes ^a	Cases (n = 366)	Controls (n = 1206)	Crude OR (95% CI)	P	Adjusted OR ^b (95% CI)	P ^b
GTAG	91 (24.86)	343 (28.44)	1.00		1.00	
GTAA	5 (1.37)	13 (1.08)	1.45 (0.50-4.17)	.491	1.47 (0.51-4.23)	.477
GTGG	22 (6.01)	62 (5.14)	1.34 (0.78-2.29)	.290	1.34 (0.78-2.30)	.288
GTGA	2 (0.55)	2 (0.17)	3.77 (0.52-27.12)	.188	3.85 (0.54-27.77)	.181
GCAG	63 (17.21)	191 (15.84)	1.24 (0.86-1.79)	.245	1.24 (0.86-1.80)	.243
GCAA	12 (3.28)	19 (1.58)	2.38 (1.12-5.08)	.025	2.40 (1.12-5.14)	.024
GCGG	44 (12.02)	113 (9.45)	1.46 (0.96-2.21)	.079	1.46 (0.96-2.22)	.076
GCGA	4 (1.09)	17 (1.41)	0.89 (0.29-2.70)	.833	0.89 (0.29-2.72)	.843
ATAG	8 (2.19)	20 (1.66)	1.51 (0.64-3.53)	.345	1.51 (0.64-3.54)	.343
ATAA	33 (9.02)	116 (9.62)	1.07 (0.68-1.68)	.761	1.08 (0.69-1.69)	.750
ATGG	7 (1.91)	28 (2.32)	0.94 (0.40-2.23)	.892	0.94 (0.40-2.23)	.891
ATGA	20 (5.46)	80 (6.63)	0.94 (0.55-1.62)	.830	0.95 (0.55-1.63)	.853
ACAG	8 (2.19)	42 (3.48)	0.72 (0.33-1.58)	.411	0.72 (0.33-1.58)	.407
ACAA	46 (12.57)	158 (13.10)	1.10 (0.73-1.64)	.650	1.10 (0.73-1.64)	.654
ACGG	1 (0.27)	1 (0.08)	3.77 (0.23-60.84)	.350	3.89 (0.24-63.07)	.340

^aThe haplotypes' order was rs57961569, rs9653226, rs13034994, and rs60226897.

^bObtained in logistic regression models with adjustment for age and gender.

on Wilms tumor may be related to RA signaling pathway.³⁵ The contradiction of results may be caused by the small sample size and racial differences.

Moreover, a large number of studies have demonstrated that the *MYCN* gene is closely related to neuroblastoma.³⁶⁻⁴¹ A study by Zaatiti et al indicated that neuroblastoma IMR-32 cell lines had significantly decreased proliferation ability after *MYCN* knockout.⁴² *MYCN* not only is related to the development of neuroblastoma, but also is one of the most powerful prognostic markers of neuroblastoma. We have delved the association between *MYCN* gene SNPs and neuroblastoma susceptibility before, and we found that SNP rs57961569 G > A was significantly associated with neuroblastoma risk.²⁵ *MYCN* also regulated the regulatory circuits of genes involved in the progression of neuroblastoma through TFAP4.⁴³ Moreover, a study by Liu et al found that *MYCN* was involved in the malignant characteristics of erythroleukemia by inhibiting the activation of p21.⁴⁴ What's more, amplified *MYCN* gene was observed in rhabdomyosarcoma,⁴⁵ pediatric T-cell acute lymphoblastic leukemia,⁴⁶ astrocytoma, and meningioma.⁴⁷

Our study is the first investigation to explore the relationship of *MYCN* gene SNPs with Wilms tumor susceptibility. There are several flaws in our research. First, only four genotypes were included in our research. The association between other *MYCN* polymorphisms and Wilms tumor susceptibility should be further explored. Secondly, the relatively small sample size, because of the low incidence of Wilms tumors, led to limited statistical power. Additionally, the environmental factors or environment-genes interaction were not considered in this study.

In conclusion, no significant association was found between *MYCN* gene polymorphisms and Wilms tumor risk in overall analysis, but stratification and haplotype analyses suggested that *MYCN* gene polymorphisms might be low penetrant variants in Wilms tumor susceptibility. Studies with larger sample size are need to verify our finding.

ACKNOWLEDGMENTS

This study was funded by grants from the Pearl River S&T Nova Program of Guangzhou (No: 201710010086), Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease (No: 2019B030301004), Scientific Research Foundation of Wenzhou (No: 2015Y0492), and Zhejiang Provincial Science and Technology Animal Experimental Platform Project (No: 016C37113).

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Huang X, Zhao J, Zhu J, et al. MYCN gene polymorphisms and Wilms tumor susceptibility in Chinese children. *J Clin Lab Anal*. 2019;33:e22988. <https://doi.org/10.1002/jcla.22988>