Original Article

Serum Interleukin-6 Level and the rs1800795 Polymorphism in its Gene Associated with Neuroblastoma Risk in Chinese Children

Qian Zhao, Mei Jin, Da-Wei Zhang, Wen Zhao, Xi-Si Wang, Zhi-Xia Yue, Chao Duan, Cheng Huang, Xiao-Li Ma

Beijing Key Laboratory of Pediatric Hematology Oncology, National Key Discipline of Pediatrics (Capital Medical University), Key Laboratory of Major Diseases in Children, Ministry of Education, Department of Hematology Oncology Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China

Abstract

Background: The pro-inflammatory cytokine, interleukin-6 (IL-6), stimulates the metastasis of several neoplasms. An association of its serum level and the single nucleotide polymorphism (SNP) rs1800795 with neuroblastoma (NB) has been reported in American and Italian cohorts. This study was to clarify whether the same association exists in Chinese children.

Methods: A total of 130 NB patients, with 77 boys (59%), 53 girls (41%), mean age 41 ± 5 months, were assigned to two groups: high risk (HR) versus intermediate-low risk (non-HR), and 50 healthy children were randomly selected as the age- and gender-matched controls. Peripheral blood samples were analyzed to determine serum IL-6 level using enzyme linked immunosorbent assay and rs1800795 SNPs phenotype using polymerase chain reaction and gene sequencing.

Results: There were 87 NB patients in the HR group and 43 NB patients in the non-HR group. A comparison of allele and genotype frequencies of the rs1800795 polymorphism between patients and controls found no association with NB risk ($P > 0.05$). The frequency of GG+GC genotype was higher in HR-NB patients than in non-HR-NB patients (64.4% vs. 48.8%, $P = 0.02$), and serum IL-6 level was much higher in HR-NB patients with GG+GC genotype than in HR-NB patients with CC genotype (4.36 ± 1.1 pg/ml vs. 1.83 ± 0.5 pg/ml; $P = 0.02$), but not in Non-HR-NB patients.

Conclusions: The polymorphism rs1800795 is associated with serum IL-6 level and level of NB risk. GG genotype might indicate that the tumor is highly malignant (prone to metastasis) and associated with poor prognosis.

Key words: Children; Interleukin-6; Neuroblastoma; Single Nucleotide Polymorphism

Introduction

Neuroblastoma (NB), the most common extracranial solid tumor of childhood, accounts for 8–10% of all childhood cancers and is responsible for about 15% of all pediatric oncology-related deaths.[1,2] Although the etiology and pathogenesis of NB are largely unknown, accumulating evidence shows that genetic polymorphisms contribute to NB susceptibility. Jin *et al*.[3] reported that the single nucleotide polymorphisms (SNPs) rs11669203 in *TGFBR3L* is associated with the risk of NB in a Chinese population. Mossé *et al*.[4] reported that individuals carrying germline mutations in *PHOX2B* are genetically predisposed to NB. The Genome-wide Association Study demonstrated that genetic variations in the *CASC15*, *BARD1*, *LMO1*, *DUSP12*, *HSD17B12*, *HACE1*, and *LIN28B* genes are associated with NB risk in North American patients of European descent.[5-9]

Interleukin-6 (IL-6) facilitates the progression of several cancers[10-13] by affecting several biological mechanisms

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.230719

Address for corresponding: Dr. Xiao-Li Ma, Beijing Key Laboratory of Pediatric Hematology Oncology; National Key Discipline of Pediatrics (Capital Medical University), Key Laboratory of Major Diseases in Children, Ministry of Education, Department of Hematology Oncology Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, , Beijing 100045, China
E-Mail: mxl1123@vip.sina.com

Received: 05-01-2018 **Edited by:** Yi Cui

How to cite this article: Zhao Q, Jin M, Zhang DW, Zhao W, Wang XS, Yue ZX, Duan C, Huang C, Ma XL. Serum Interleukin-6 Level and the rs1800795 Polymorphism in its Gene Associated with Neuroblastoma Risk in Chinese Children. Chin Med J 2018;131:1075-8.

and cellular processes, including apoptosis, survival, proliferation, angiogenesis, invasiveness, metastasis, and metabolism.[14] Elevated serum IL-6 level has been shown to correlate with NB progression and development,[15] and to promote growth and survival of NB cells in the bone marrow.[16] In recent years, reports have shown that the polymorphism rs1800795, which is located in the IL-6 promoter region, affects the constitutive transcription of the IL-6 gene, thereby controlling the level of IL-6. Results showing a close relationship of rs1800795 SNP to NB are inconsistent.[17,18] Furthermore, none of these reports has dealt with Chinese NB patients. Consequently, we conducted a case-control study to examine the relationship of IL-6 level and rs1800795 SNP status to the risk of NB in China.

Methods

Ethics statement

This study is approved by the Research Ethics Committee at the Beijing Children's Hospital and written informed consent was obtained by all children's legal guardians according to the *Declaration of Helsinki*.

Study population

A total of 130 patients with histopathologically confirmed NB were recruited from the Beijing Children's Hospital between August 2012 and August 2015 (77 boys, 53 girls; mean age: 41 ± 5 months). The NB for each patient was staged by at least two pathologists according to the International Neuroblastoma Staging System. During the same period, 50 healthy children were randomly selected as age- and gender-matched controls after receiving a routine physical examination (28 boys, 22 girls; mean age: 39 ± 6 months). Both the cases and the controls were unrelated ethnic Chinese Han individuals, and all were born in North China. Clinical and biologic characteristics of the patients are shown in Table 1. Patients were assigned to two risk groups based on the Children's Oncology Group risk assignment algorithm.[19]

Patient samples

Peripheral blood samples were drawn in ethylenediaminetetraacetic acid-containing vacuum tubes from 130 patients with NB when newly diagnosed before any treatment, and 50 cancer-free Chinese children selected as sex- and age-matched normal controls after a fast of more than 8 h. Genomic DNA was extracted from peripheral blood leukocytes using a commercial DNA isolation kit according to the manufacturer's instructions. The IL-6 rs1800795 polymorphism was genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the Sequenom MassARRAY according to the manufacturer's instructions. The forward and reverse genotyping primers were: 5'-ATGCCAAGTGCTGAGTCACTA-3' and 5'-TCGAGGGCAGAATGAGCCTC-3'.

Serum levels of IL-6 were determined in triplicate samples by ELISA according to the manufacturer's protocol (Quantikine immune assay kit, R&D Systems, Minneapolis, MN, USA).

Statistical analysis

To evaluate the potential deviation from Hardy-Weinberg equilibrium, we compared the obtained and expected allele frequencies in the case and control samples using a Chi-square test. To evaluate the association between cases or controls and the presence of polymorphism, we used unconditional multiple logistic regression to estimate odds ratios (*OR*s) with 95% confidence intervals (*CI*s). To decide whether to accept or reject the null hypothesis, we used the likelihood ratio statistic with 95% *CI* ($P < 0.05$) calculated for adjusted *OR*. Statistical analyses were carried out using SPSS 16.0 software (IBM Corp., Armonk, NY, USA). A $P < 0.05$ was considered as statistically significant.

Results

Characteristics and clinical features

As shown in Table 1, the current study included 130 cases of NB and 50 age-, gender-, and ethnicity-matched controls. The patients were treated and followed up at Beijing Children's Hospital. In the NB group, the primary tumors were in the retroperitoneal space in 111 cases (85.4%) and posterior mediastinum in 19 cases (14.6%). The metastases were in the bone marrow in 84 patients (64.6%), bone in 52 patients (40.0%), liver in 5 patients (3.8%), skin in 3 patients (2.3%), and lung in 1 patient (0.7%). The *MYCN* was amplified in 11 patients and nonamplified in 47 patients.

Relationship of rs1800795 single nucleotide polymorphisms between neuroblastoma cases and controls

A summary of the genotype and allele distribution of the rs1800795 polymorphisms in the NB patients and controls is shown in Table 2. The genotype and allele frequencies of the rs1800795 locus were similar between NB patients and controls ($P > 0.05$).

Relationship between the rs1800795 single nucleotide polymorphisms and neuroblastoma risk and *MYCN* gene amplification status

The GG+GC genotype frequency was compared to CC genotype frequency in NB children with different NB risk level and different *MYCN* gene amplification status.

Table 1: Characteristics and clinical features of all subjects, *n* (%)

Characteristics	Controls ($n = 50$)	NB patients ($n = 130$)
Gender		
Boy	28 (56.0)	77 (59.2)
Girl	22 (44.0)	53 (40.8)
Age		
≤18 months	19 (38.0)	34 (26.2)
>18 months	31 (62.0)	96 (73.8)
Risk		
HR		87 (66.9)
Non-HR		43 (33.1)

HR: High risk; NB: Neuroblastoma.

Table 3 shows that frequency of GG+GC genotype was significantly higher in the high risk (HR) group ($P < 0.05$) but similar in groups categorized by *MYCN* amplification status ($P > 0.05$).

Relationship between rs1800795 single nucleotide polymorphisms and serum interleukin-6 level in different neuroblastoma-risk groups and *MYCN* gene amplification status groups

In high NB risk patients (the HR group), serum IL-6 level was obviously higher in the GG+GC genotype group than in the CC genotype group (4.36 ± 1.10 pg/ml vs. 1.83 ± 0.50 pg/ml; $P = 0.02$), while no obvious difference was found in the non-HR group (2.13 ± 0.80 pg/ml vs. 1.53 ± 0.60 pg/ml; $P = 0.19$). In addition, serum IL-6 level was similar between the genotypes with amplified *MYCN* gene (2.56 ± 0.70 pg/ml vs. 2.05 ± 0.90 pg/ml; $P = 0.16$) and nonamplified *MYCN* gene (2.81 ± 0.60 pg/ml vs. 1.26 ± 0.50 pg/ml; $P = 0.27$).

Discussion

Previous studies have linked rs1800795 SNP in the IL-6 promoter to the occurrence of a variety of tumors.[20] Some recent studies have focused on the relationship of rs1800795 SNP to the occurrence of childhood NB. Lagmay *et al*.[17] showed that the GG genotype of the IL-6 rs1800795 SNP is responsible for the worse outcome in patients with HR for NB. However, Totaro *et al*.[18] stated that this SNP does not predispose to NB development but is associated with NB progression. They found that Italian patients with NB who were homozygous for the C allele had worse outcomes than patients who were homozygous or heterozygous for the G allele. The data from our genetic study to assess the association between IL-6 rs1800795 SNP and NB in Chinese children suggested that this SNP does not predispose to NB development but is associated with the level of NB risk. In our Chinese NB patients, the ratio of GG+GC genotype was higher in the HR-NB group than non-HR-NB group, which is consistent with Lagmay's conclusion. Racial differences might account for the differences between results; so, further investigations with larger samples of patients are needed.

The *MYCN* gene codes for an oncogenic transcription factor and belongs to the MYC family of genes. *MYCN* gene is amplified in approximately 20% of all NB cases, and amplified *MYCN* gene is considered to be a molecular marker identifying individuals with HR of NB. *MYCN* gene was amplified in 11 of our 130 NB patients, but nonamplified in 47 patients. *MYCN* gene amplification status was unavailable in the remaining 72 patients. Our study aimed to determine whether *MYCN* gene amplification and the IL-6 SNP of rs1800795 have a synergistic relationship but found no combination of factors significantly affected serum IL-6 level.

Many recent articles have identified the elevated level of circulating IL-6 as a marker of poor prognosis in various cancers, including NB. In Egler's research,[15] serum IL-6 level was higher in patients with HR-NB than in those with non-HR-NB. Furthermore, in our research with a focus on the serum IL-6 level–rs1800795 SNP relationship, IL-6 level was higher in HR-NB patients with GG+GC genotype than in HR-NB patients with CC genotype, which is consistent with the conclusion of Fishman *et al*.[21]

In conclusion in our research, the SNP rs1800795 was associated with serum IL-6 level and level of NB risk. GG genotype might indicate that a tumor is highly malignant (prone to spread) and associated with poor prognosis. Since rs1800795 is located in the promoter region of IL-6, we suggest that genetic variants of rs1800795 might influence serum IL-6 level. However, additional investigations are needed to confirm this hypothesis.

Table 2: Case-control study of rs1800795 SNP, *n* (%)

Items	Controls (*n* = 50)	NB patients (*n* = 130)	*P*	*OR* (95% *CI*)
Genotype				
GG	26 (52.0)	77 (59.2)	–	–
GC	19 (38.0)	42 (32.3)	0.26	1.29 (0.87–1.91)
CC	5 (10.0)	11 (8.4)	0.12	1.35 (0.96–1.92)
GG/GC	45 (90.0)	119 (91.5)	–	–
CC	5 (10.0)	11 (8.4)	0.09	1.56 (0.89–2.04)
Allele				
G	71 (71.0)	196 (75.0)	–	–
C	29 (29.0)	64 (25.0)	0.07	1.61 (0.87–2.21)

NB: Neuroblastoma; *OR*: Odds ratio; *CI*: Confidence interval; SNP: Single nucleotide polymorphism; –: Not applicable.

Table 3: Relationship between rs1800795 SNP and NB risk and *MYCN* status

Items	*n*	GG + GC genotype, *n* (%)	CC genotype, *n* (%)
Risk			
HR	87	56 (64.4)	31 (35.6)
Non-HR	43	21 (48.8)	22 (51.2)
P		0.02	0.56
MYCN			
Amplified	11	2 (18.2)	9 (81.8)
Nonamplified	47	11 (23.4)	36 (76.6)
NA	72	22 (30.6)	50 (69.4)
*P**		0.16	0.25

*Compared in amplified and nonamplified group. NA: Not available; NB: Neuroblastoma; HR: High risk; SNP: Single nucleotide polymorphism.

Financial support and sponsorship

This work was supported in part by a grant from Beijing Municipal Commission of Science and Technology Capital Clinical Medicine Applied Research and Popularization Special Funding (No. Z151100004015159).

Conflicts of interest

There are no conflicts of interest.

References

1. Schulte JH, Eggert A. Neuroblastoma. Crit Rev Oncog 2015;20:245-70. doi: 10.1615/CritRevOncog.2015014033.
2. Luksch R, Castellani MR, Collini P, De Bernardi B, Conte M, Gambini C, *et al.* Neuroblastoma (peripheral neuroblastic tumours). Crit Rev Oncol Hematol 2016;107:163-81. doi: 10.1016/j.critrevonc.2016.10.001.
3. Jin Y, Wang H, Han W, Lu J, Chu P, Han S, *et al.* Single nucleotide polymorphism rs11669203 in TGFBR3L is associated with the risk of neuroblastoma in a Chinese population. Tumour Biol 2016;37:3739-47. doi: 10.1007/s13277-015-4192-6.
4. Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, *et al.* Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 2008;455:930-5. doi: 10.1038/nature07261.
5. Capasso M, Diskin S, Cimmino F, Acierno G, Totaro F, Petrosino G, *et al.* Common genetic variants in NEFL influence gene expression and neuroblastoma risk. Cancer Res 2014;74:6913-24. doi: 10.1158/0008-5472.CAN-14-0431.
6. Maris JM, Mosse YP, Bradfield JP, Hou C, Monni S, Scott RH, *et al.* Chromosome 6p22 locus associated with clinically aggressive neuroblastoma. N Engl J Med 2008;358:2585-93. doi: 10.1056/NEJMoa0708698.
7. Capasso M, Devoto M, Hou C, Asgharzadeh S, Glessner JT, Attiyeh EF, *et al.* Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. Nat Genet 2009;41:718-23. doi: 10.1038/ng.374.
8. Wang K, Diskin SJ, Zhang H, Attiyeh EF, Winter C, Hou C, *et al.* Integrative genomics identifies LMO1 as a neuroblastoma oncogene. Nature 2011;469:216-20. doi: 10.1038/nature09609.
9. Diskin SJ, Capasso M, Schnepp RW, Cole KA, Attiyeh EF, Hou C, *et al.* Common variation at 6q16 within HACE1 and LIN28B influences susceptibility to neuroblastoma. Nat Genet 2012;44:1126-30. doi: 10.1038/ng.2387.
10. Gomes M, Coelho A, Araújo A, Azevedo A, Teixeira AL, Catarino R, *et al.* IL-6 polymorphism in non-small cell lung cancer: A prognostic value? Tumour Biol 2015;36:3679-84. doi: 10.1007/s13277-014-3006-6.
11. Dethlefsen C, Højfeldt G, Hojman P. The role of intratumoral and systemic IL-6 in breast cancer. Breast Cancer Res Treat 2013;138:657-64. doi: 10.1007/s10549-013-2488-z.
12. Waldner MJ, Foersch S, Neurath MF. Interleukin-6 – A key regulator of colorectal cancer development. Int J Biol Sci 2012;8:1248-53. doi: 10.7150/ijbs.4614.
13. Macciò A, Madeddu C. The role of interleukin-6 in the evolution of ovarian cancer: Clinical and prognostic implications – A review. J Mol Med (Berl) 2013;91:1355-68. doi: 10.1007/s00109-013-1080-7.
14. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol 2016;37:11553-72. doi: 10.1007/s13277-016-5098-7.
15. Egler RA, Burlingame SM, Nuchtern JG, Russell HV. Interleukin-6 and soluble interleukin-6 receptor levels as markers of disease extent and prognosis in neuroblastoma. Clin Cancer Res 2008;14:7028-34. doi: 10.1158/1078-0432.CCR-07-5017.
16. Ara T, Song L, Shimada H, Keshelava N, Russell HV, Metelitsa LS, *et al.* Interleukin-6 in the bone marrow microenvironment promotes the growth and survival of neuroblastoma cells. Cancer Res 2009;69:329-37. doi: 10.1158/0008-5472.CAN-08-0613.
17. Lagmay JP, London WB, Gross TG, Termuhlen A, Sullivan N, Axel A, *et al.* Prognostic significance of interleukin-6 single nucleotide polymorphism genotypes in neuroblastoma: rs1800795 (promoter) and rs8192284 (receptor). Clin Cancer Res 2009;15:5234-9. doi: 10.1158/1078-0432.CCR-08-2953.
18. Totaro F, Cimmino F, Pignataro P, Acierno G, De Mariano M, Longo L, *et al.* Impact of interleukin-6 -174 G>C gene promoter polymorphism on neuroblastoma. PLoS One 2013;8:e76810. doi: 10.1371/journal.pone.0076810.
19. Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, *et a1*. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol 2015;33:3008-17. doi: 10.1200/JCO.2014.59.4648.
20. Xu B, Niu XB, Wang ZD, Cheng W, Tong N, Mi YY, *et al.* IL-6 -174G>C polymorphism and cancer risk: A meta-analysis involving 29,377 cases and 37,739 controls. Mol Biol Rep 2011;38:2589-96. doi: 10.1007/s11033-010-0399-1.
21. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, *et al.* The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76. doi: 10.1172/JCI2629.

外周血白细胞介素-6水平及其rs1800795位点基因单核苷酸多态性与儿童神经母细胞瘤的相关性研究

摘要

目的：白细胞介素-6（IL-6）是一种促炎性细胞因子，对肿瘤的转移有促进作用。有研究显示IL-6及其单核苷酸多态性(single nucleotide polymorphisms, SNP) rs1800795与美国和意大利的人群中的神经母细胞瘤（NB）有关。本文主要探讨外周血IL-6水平及其rs1800795位点SNP与中国NB儿童之间的相关性。

方法：收集我院诊治的NB患儿共130例，分为两组(中低危组及高危组)，及同期年龄和性别匹配的健康儿童50例作为对照组，应用PCR和基因测序的方法，分析L-6水平及其rs1800795位点的SNP与儿童NB的危险度和预后之间的关系。

结果：NB组共有男孩77例(59%)和女孩53例(41%)，平均年龄41±5个月，其中高危组患儿87例，中低危组患儿43例。Rs1800795位点的各基因型及等位基因，在NB患儿及正常儿童中的分布均无明显差异（P>0.05）；在高危组患儿中GG + GC基因型频率高于非高危患儿（64.4% vs. 48.8%, $P = 0.02$），而高危患儿中，伴GG + GC基因型的血清IL-6水平比CC基因型高（4.36 ± 1.1 pg/ml vs. 1.83 ± 0.5 pg/ml; $P = 0.02$），但非高危患儿无此现象。

结论：外周血IL-6水平及rs1800795位点的基因多态性与NB的危险度及预后相关。GG基因型可能提示肿瘤高度恶性，易转移，预后差。