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ORIGINAL RESEARCH

Common variations within *HACE1* gene and neuroblastoma susceptibility in a Southern Chinese population

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Abstract: Neuroblastoma is a common fatal pediatric cancer of the developing sympathetic nervous system, which accounts for ~10% of all pediatric cancer deaths. To investigate genetic risk factors related to neuroblastoma, many genome-wide association studies have been performed, and single nucleotide polymorphisms (SNPs) within *HACE1* gene have been identified to associate with neuroblastoma risk. However, the association of the *HACE1* SNPs with neuroblastoma needs to be validated in Southern Chinese children. We genotyped five SNPs located in the *HACE1* gene (rs4336470 C>T, rs9404576 T>G, rs4079063 A>G, rs2499663 T>C, and rs2499667 A>G) in 256 Southern Chinese patients in comparison with 531 ethnically matched healthy controls. Single locus analysis showed no significant association between any of *HACE1* SNPs and neuroblastoma risk in Southern Chinese children. However, when all the risk genotypes were combined, we found a borderline significant trend toward an increased neuroblastoma risk with 4–5 risk genotypes (adjusted odds ratio =1.36, 95% confidence interval =0.98–1.89, P=0.065). Moreover, stratified analysis found that carriers of 4–5 risk genotypes tended to develop neuroblastoma in the retroperitoneal region and have more aggressive tumors, progressing to advanced clinical stages III/IV, when compared with those of 0–3 risk genotypes. In conclusion, *HACE1* gene may have weak effect on neuroblastoma risk in Southern Chinese children. Large well-designed studies are needed to strengthen our findings.

Keywords: *HACE1*, susceptibility, neuroblastoma, GWAS, polymorphism

Introduction

Neuroblastoma, a severe malignancy of the developing sympathetic nervous system, has been recognized as the most common extracranial solid cancer in infancy, accounting for ~7%–10% of all childhood cancers.[1–3] Despite advanced therapies and marked improvements in the cure rates for many childhood cancers, the mortality of neuroblastoma remains high. It constitutes ~10% of all pediatric cancer-related deaths.[4,5] The incidence rate of neuroblastoma in the live births is ~7.7 cases per million in China,[6] which is lower than that in the USA.[7] Generally, <40% of neuroblastoma patients survive >5 years after diagnosis. Moreover, survivors are likely to have fewer chances for employment, marriage, and high income because of their chronic health conditions.[8] Therefore, neuroblastoma has become a great burden and challenge to their families and public health, a situation that warrants further improvement.[9,10]

Epidemiology studies searching for risk factors failed to identify common environmental exposures that can affect the development of neuroblastoma.[11,12] However, accumulating evidence from genome-wide association studies (GWASs) suggests

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that genetic factors are able to modify neuroblastoma susceptibility.[13,14] A recent GWAS by Diskin et al[15] has demonstrated that several loci are associated with neuroblastoma susceptibility and disease progression, such as loci within the *HACE1* (encoding HECT domain- and ankyrin repeat-containing E3 ubiquitin protein ligase 1) and *LIN28B* (encoding lin28 homolog B) genes. In that study, 2,817 neuroblastoma cases and 7,473 controls were enrolled, and low HACE1 expression was observed to be significantly associated with worse overall survival in newly diagnosed neuroblastoma patients, suggesting *HACE1* at chromosome 6q16 as a tumor suppressor gene. In addition, the authors identified five single nucleotide polymorphisms (SNPs) (rs4336470 C>T, rs9404576 T>G, rs4079063 A>G, rs2499663 T>C, and rs2499667 A>G) within the *HACE1* gene that may contribute independently to neuroblastoma risk. To date, the association between neuroblastoma susceptibility and these SNPs has been validated in the European ancestry, African-Americans, and Italian population[15,16] but not yet in Asians.

To corroborate and comprehensively evaluate the impact of the GWAS-identified *HACE1* gene polymorphisms on neuroblastoma risk, these five SNPs were analyzed in a Southern Chinese population with 256 neuroblastoma cases and 531 cancer-free controls.

Subjects and methods

Study subjects

A total of 256 histopathologically confirmed primary neuroblastoma cases and 531 cancer-free controls were included in this study, as we had described in detail previously.[10,17–20] Briefly, all the 256 neuroblastoma cases were newly diagnosed and histopathologically confirmed patients without metastasis from other organs. The cases were genetically unrelated ethnic Han Chinese children who received treatments at the Department of Pediatric Surgery, Guangzhou Women and Children's Medical Center, mainly between February 2010 and November 2015, while age-, gender-, and race-matched controls were randomly recruited from children undergoing routine physical examination at the same hospital during the same period. The parents or guardians of the children provided informed consent for the children's participation in this study. This study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center.

Genotyping

Genotyping for the five GWAS-identified polymorphisms (rs4336470 C>T, rs9404576 T>G, rs4079063 A>G, rs2499663 T>C, and rs2499667 A>G)[15] was performed in a 384-well plate using TaqMan Real-Time PCR method using the typical 7900 HT sequence detector system (Applied Biosystems, Foster City, CA, USA) as described previously.[21,22] Approximately 10% of the samples were randomly selected and regenotyped to validate the accuracy of genotyping results from TaqMan Real-Time PCR. The results were 100% concordant.

Statistical analysis

The chi-square test was performed to examine the differences in the demographics and frequency distributions of genotypes between cases and controls. Unconditional multivariate logistic regression analysis was performed and adjusted for age and gender. The strength of associations between these five polymorphisms and neuroblastoma risk was estimated using odds ratios (ORs) and 95% confidence intervals (CIs). Stratified analysis was performed by age, gender, tumor sites, and clinical stages. *P*-values <0.05 were considered as statistically significant. All statistical analyses were two-sided and performed using the SAS software (version 9.1; SAS Institute, Cary, NC, USA).

Results

Population characteristics

The distributions of the demographic characteristics of the cases and controls are summarized in Table S1. No statistically significant difference was observed between cases and controls regarding age (P=0.239) and gender (P=0.333). According to International Neuroblastoma Staging System criteria,[23] 54 (21.09%), 65 (25.39%), 44 (17.19%), 77 (30.08%), and 9 (3.52%) patients had clinical stage I, II, III, IV, and 4s neuroblastomas, respectively. In terms of tumor sites, the neuroblastoma mainly occurred in adrenal glands (n=46, 17.97%), retroperitoneal regions (n=87, 33.98%), mediastinum (n=90, 35.16%), and other regions (n=25, 9.77%).

Associations of selected *HACE1* gene SNPs with neuroblastoma susceptibility

The genotype frequencies of the five selected SNPs and their associations with the risk of neuroblastoma are shown in Table 1. Of the included participants, 249 cases and 530 controls were successfully genotyped. Overall, the association between individual polymorphisms and neuroblastoma susceptibility did not reach statistical significance. We found that the rs4336470 T, rs9404576 G, rs4079063 A, rs2499663 T, and rs2499667 A allele carriers were associated with an increased neuroblastoma risk. When the risk genotypes were combined, we observed a borderline increased

Table 1 Logistic regression analysis of associations of *HACE1* gene polymorphisms with neuroblastoma susceptibility

Genotypes	Cases (n=249), No. (%)	Controls (n=530), No. (%)	*P*-value[a]	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)[b]	*P*-value[b]
rs4336470 C>T							
CC	130 (52.21)	303 (57.17)		1.00		1.00	
CT	99 (39.76)	188 (35.47)		1.23 (0.89–1.69)	0.207	1.22 (0.89–1.68)	0.220
TT	20 (8.03)	39 (7.36)		1.20 (0.67–2.13)	0.545	1.22 (0.68–2.18)	0.500
Dominant	119 (47.79)	227 (42.83)	0.194	1.22 (0.90–1.65)	0.194	1.22 (0.90–1.65)	0.197
Additive model			0.429	1.15 (0.91–1.45)	0.247	1.15 (0.91–1.46)	0.236
Recessive	229 (91.97)	491 (92.64)	0.742	1.10 (0.63–1.93)	0.740	1.13 (0.64–1.98)	0.681
rs9404576 T>G							
TT	134 (53.82)	303 (57.17)		1.00		1.00	
TG	97 (38.96)	189 (35.66)		1.16 (0.84–1.60)	0.359	1.16 (0.84–1.59)	0.373
GG	18 (7.23)	38 (7.17)		1.07 (0.59–1.95)	0.822	1.09 (0.60–1.99)	0.774
Dominant	115 (46.18)	227 (42.83)	0.379	1.15 (0.85–1.55)	0.379	1.15 (0.85–1.55)	0.380
Additive model			0.657	1.09 (0.86–1.38)	0.479	1.09 (0.86–1.39)	0.462
Recessive	231 (92.77)	492 (92.83)	0.976	1.01 (0.56–1.81)	0.976	1.03 (0.57–1.85)	0.921
rs4079063 A>G							
AA	92 (36.95)	189 (35.66)		1.00		1.00	
AG	116 (46.59)	242 (45.66)		0.99 (0.71–1.37)	0.928	0.98 (0.70–1.36)	0.880
GG	41 (16.47)	99 (18.68)		0.85 (0.55–1.32)	0.472	0.85 (0.55–1.32)	0.469
Dominant	157 (63.05)	341 (64.34)	0.727	0.95 (0.69–1.29)	0.726	0.94 (0.69–1.28)	0.690
Additive model			0.749	0.93 (0.76–1.15)	0.523	0.93 (0.75–1.15)	0.509
Recessive	208 (83.53)	431 (81.32)	0.450	0.86 (0.58–1.28)	0.453	0.86 (0.58–1.29)	0.466
rs2499663 T>C							
TT	93 (37.35)	189 (35.66)		1.00		1.00	
TC	115 (46.18)	243 (45.85)		0.96 (0.69–1.34)	0.819	0.95 (0.68–1.33)	0.773
CC	41 (16.47)	98 (18.49)		0.85 (0.55–1.32)	0.471	0.85 (0.55–1.32)	0.470
Dominant	156 (62.65)	341 (64.34)	0.648	0.93 (0.68–1.27)	0.647	0.92 (0.68–1.26)	0.614
Additive model			0.767	0.93 (0.75–1.15)	0.497	0.93 (0.75–1.15)	0.486
Recessive	208 (83.53)	432 (81.51)	0.489	0.87 (0.58–1.30)	0.675	0.87 (0.59–1.30)	0.508
rs2499667 A>G							
AA	90 (36.14)	181 (34.15)		1.00		1.00	
AG	118 (47.39)	248 (46.79)		0.96 (0.69–1.34)	0.796	0.95 (0.68–1.32)	0.744
GG	41 (16.47)	101 (19.06)		0.82 (0.53–1.27)	0.369	0.81 (0.52–1.27)	0.362
Dominant	159 (63.86)	349 (65.85)	0.587	0.92 (0.67–1.26)	0.586	0.91 (0.66–1.24)	0.546
Additive model			0.657	0.91 (0.74–1.13)	0.400	0.91 (0.73–1.13)	0.384
Recessive	208 (83.53)	429 (80.94)	0.379	0.84 (0.56–1.25)	0.383	0.84 (0.56–1.25)	0.394
Risk genotypes							
0–3	167 (67.07)	390 (73.58)		1.00		1.00	
4–5	82 (32.93)	140 (26.42)	0.062	1.37 (0.99–1.90)	0.061	1.36 (0.98–1.89)	0.065

Notes: [a]Chi-square test for genotype distributions between cases and controls. [b]Adjusted for age and gender.
Abbreviations: CI, confidence interval; OR, odds ratio.

neuroblastoma risk for the subjects carrying 4–5 risk genotypes (adjusted OR =1.36, 95% CI =0.98–1.89, *P*=0.065) when compared with those carrying 0–3 risk genotypes.

Stratified analysis of selected polymorphisms and neuroblastoma susceptibility

We performed stratification analysis on rs4336470 C>T and rs9404576 T>G to estimate the effects of variant genotypes with neuroblastoma susceptibility. The cumulative effects of the five risk genotypes were also determined (Table 2). Similarly, as described earlier, no significant association was obtained in our study. However, a comparison of 0–3 combined risk genotypes and 4–5 combined risk genotypes indicated that 4–5 combined risk genotypes had a trend to increase the risk of clinical stages III/IV neuroblastoma (adjusted OR =1.51, 95% CI =0.98–2.31, *P*=0.060) and the risk of tumor in retroperitoneal region (adjusted OR =1.55, 95% CI =0.94–2.54, *P*=0.083).

Discussion

The *HACE1* gene encodes an E3 ubiquitin protein ligase, which was first identified in human Wilms' tumor and further observed to be silenced in the majority of Wilms' tumors via hypermethylation.[24] Similarly, a marked reduction in *HACE1* gene expression or even epigenetic silencing caused

Table 2 Stratification analysis for associations of *HACE1* gene polymorphisms with neuroblastoma susceptibility

Variables	rs4336470 (cases/controls)		Adjusted OR (95% CI)	*P*-value[a]	rs9404576 (cases/controls)		Adjusted OR (95% CI)	*P*-value[a]	Risk genotype (cases/controls)		Adjusted OR (95% CI)	*P*-value[a]
	CC	CT/TT			TT	TG/GG			0–3	4–5		
Age, months												
≤ 18	54/131	44/101	1.06 (0.66–1.71)	0.809	54/131	44/101	1.06 (0.66–1.71)	0.806	69/178	29/54	1.39 (0.82–2.37)	0.223
> 18	76/172	75/126	1.33 (0.90–1.98)	0.154	80/172	71/126	1.20 (0.81–1.78)	0.374	98/212	53/86	1.32 (0.87–2.01)	0.192
Gender												
Female	52/140	49/92	1.45 (0.90–2.33)	0.124	53/140	48/92	1.40 (0.87–2.24)	0.169	70/173	31/59	1.30 (0.78–2.18)	0.315
Male	78/163	70/135	1.08 (0.73–1.60)	0.702	81/163	67/135	1.00 (0.67–1.48)	0.982	97/217	51/81	1.40 (0.92–2.14)	0.121
Sites of origin												
Adrenal gland	24/303	22/227	1.23 (0.67–2.26)	0.506	23/303	23/227	1.35 (0.73–2.47)	0.337	31/390	15/140	1.34 (0.70–2.57)	0.375
Retroperitoneal	40/303	41/227	1.36 (0.85–2.18)	0.196	41/303	40/227	1.30 (0.81–2.08)	0.275	52/390	29/140	1.55 (0.94–2.54)	0.083
Mediastinum	47/303	43/227	1.23 (0.79–1.93)	0.362	50/303	40/227	1.08 (0.69–1.69)	0.746	61/390	29/140	1.33 (0.82–2.16)	0.242
Others	14/303	10/227	0.93 (0.40–2.13)	0.855	15/303	9/227	0.78 (0.33–1.81)	0.558	18/390	6/140	0.94 (0.37–2.43)	0.904
Clinical stages												
I + II +4s	60/303	58/227	1.29 (0.87–1.93)	0.211	63/303	55/227	1.16 (0.78–1.74)	0.458	79/390	39/140	1.39 (0.90–2.14)	0.135
III + IV	61/303	57/227	1.31 (0.87–1.96)	0.197	62/303	56/227	1.26 (0.84–1.90)	0.259	77/390	41/140	1.51 (0.98–2.31)	0.060

Note: [a]Adjusted for age and gender.
Abbreviations: CI, confidence interval; OR, odds ratio.

by methylation has been reported in colorectal carcinoma, gastric cancer, breast malignancy, and nasal-type extranodal NK/T-cell lymphoma.[25–28] HACE1 depletion enhances cell migration independently of growth factor stimulation, which may promote malignant conversion. HACE1 inhibits cell migration by degrading small GTPase Rac1 (a key regulator of cell motility)[29] and suppresses cell growth through its E3 ubiquitin ligase function. Downregulation of HACE1 is a common event in multiple human tumors. HACE1 inactivation in mice leads to the development of cancer, a process that is accelerated with the addition of "second hits" such as mutations in p53.[30] Moreover, HACE1 inhibits cell cycle progression and regulates ligand-activated transcription by regulating cyclin D1 degradation[30] and retinoic acid receptor (RAR) activity,[31] respectively. Taken together, HACE1 is considered as a putative tumor suppressor. HACE1 deficiency or downregulation may increase the susceptibility to additional genetic or environmental cancer triggers.

To the best of our knowledge, all of the GWASs on neuroblastoma have been performed in European American populations. In fact, European Americans form a structured population due to historical immigration of diverse source populations. Therefore, with an aim to prevent false-positive associations resulting from population stratification, it is necessary to discern the ancestry of European Americans who were genotyped in the association studies.[32] Some significantly associated SNPs have been replicated in North European sample from the UK,[33] African Americans,[34] and Italian population.[15,16] Based on these observations, it is obvious that confirmatory studies are needed to validate the former GWAS findings in different populations and ethnicities.[16]

In order to validate the association between *HACE1* polymorphisms and neuroblastoma susceptibility in Southern Chinese population, we conducted the current hospital-based case–control study. Unexpectedly, the association between these five polymorphisms and neuroblastoma susceptibility did not reach statistical significance. Only when the risk genotypes were combined, we observed a borderline significantly increased neuroblastoma risk among subjects carrying 4–5 risk genotypes versus those carrying 0–3 risk genotypes. Similarly, no significant difference was obtained in the stratified analysis. However, we found that 4–5 combined risk genotypes tended to increase the risk of developing clinical stages III/IV neuroblastoma and tumor in retroperitoneal region. Diskin et al[15] report that the rs4336470 C, rs9404576 T, rs4079063 A, rs2499663 T, and rs2499667 A allele carriers may confer risk to develop

neuroblastoma. Thus, in the current study, we found that the rs4336470 T rs9404576 G, rs4079063 A, rs2499663 T, and rs2499667 A allele carriers were associated with an increased neuroblastoma risk. The rs4079063 A, rs2499663 T, and rs2499667 A allele carriers have a similar trend with the Diskin's study. Thus, the rest two have an opposite effect. This may be ascribed to the limited sample size as well as the ethnicity difference.

Although this is the first study to estimate the association between these five SNPs in *HACE1* gene and neuroblastoma susceptibility in Southern Chinese children, several limitations should be addressed. First, because of the nature of retrospective study design, information and selection bias could not be completely avoided. We could only reduce these biases through performing frequency matching of neuroblastoma cases and controls by age and gender, to some extent, since information on living environment, dietary intake, and paternal exposures was not available. Second, only five most significant polymorphisms reported previously elsewhere[15] are included in the present study. More polymorphisms, especially the potentially functional SNPs not contained in GWASs, remained to be discovered and replicated. Finally, although this is the largest study in Southern Chinese population, there are only 256 neuroblastoma patients and 531 cancer-free controls enrolled. The sample size is relatively small, which may have limited the statistical power.

Conclusion

Our results suggested that these five SNPs within *HACE1* gene were not associated with neuroblastoma susceptibility in the Southern Chinese population, but several trends of combined risk genotypes should be mentioned. Our study highlights genetic heterogeneity in neuroblastoma susceptibility in different populations. In the future, well-designed prospective studies with larger sample size and more homogeneous samples should be performed to confirm our findings.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Characteristics of neuroblastoma cases and cancer-free controls

Variables	Cases (n=256)		Controls (n=531)		*P*-value[a]
	n	%	n	%	
Age range, months	0–156		0.07–156		0.239
≤18	101	39.45	233	43.88	
>18	155	60.55	298	56.12	
Mean ± SD	30.87±26.45		29.73±24.86		
Gender					
Female	103	40.23	233	43.88	0.333
Male	153	59.77	298	56.12	
Clinical stages					
I	54	21.09			
II	65	25.39			
III	44	17.19			
IV	77	30.08			
4s	9	3.52			
NA	7	2.73			
Sites of origin					
Adrenal gland	46	17.97			
Retroperitoneal region	87	33.98			
Mediastinum	90	35.16			
Other regions	25	9.77			
NA	8	3.13			

Note: [a]Two-sided χ^2 test for distributions between neuroblastoma cases and controls.
Abbreviation: NA, not available.