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
that genetic factors are able to modify neuroblastoma susceptibility. A recent GWAS by Diskin et al 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 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. 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) 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. 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, 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
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* | rs9404576 (cases/controls) | Adjusted OR (95% CI) | P-value* | Risk genotype (cases/controls) | Adjusted OR (95% CI) | P-value* |
|-------------------------|-----------------------------|-----------------------|----------|-----------------------------|-----------------------|----------|-------------------------------|-----------------------|----------|
| Age, months             |                             |                       |          |                             |                       |          |                               |                       |          |
| ≤18                     | 54/131                      | 0.809                 | 0.139    | 54/131                      | 0.806                 | 0.374    | 69/178                        | 0.39 (0.82–2.37)       | 0.223    |
| >18                     | 76/172                      | 1.08 (0.90–1.98)      | 0.154    | 80/172                      | 1.20 (0.81–1.78)      | 0.374    | 98/212                        | 1.32 (0.87–2.01)       | 0.192    |
| Gender                  |                             |                       |          |                             |                       |          |                               |                       |          |
| Female                  | 52/140                      | 1.45 (0.90–2.31)      | 0.124    | 53/140                      | 1.40 (0.87–2.24)      | 0.169    | 70/173                        | 1.30 (0.78–2.18)       | 0.315    |
| Male                    | 78/163                      | 1.08 (0.73–1.60)      | 0.702    | 81/163                      | 1.00 (0.67–1.48)      | 0.982    | 97/217                        | 1.40 (0.92–2.14)       | 0.121    |
| Sites of origin         |                             |                       |          |                             |                       |          |                               |                       |          |
| Adrenal gland           | 24/303                      | 1.23 (0.67–2.26)      | 0.506    | 23/303                      | 1.35 (0.73–2.47)      | 0.337    | 31/390                        | 1.34 (0.70–2.57)       | 0.375    |
| Retroperitoneal         | 40/303                      | 1.36 (0.85–2.18)      | 0.196    | 41/303                      | 1.30 (0.81–2.08)      | 0.275    | 52/390                        | 1.55 (0.94–2.54)       | 0.083    |
| Mediastinum             | 47/303                      | 1.23 (0.79–1.93)      | 0.362    | 50/303                      | 1.08 (0.69–1.69)      | 0.746    | 61/390                        | 1.33 (0.82–2.16)       | 0.242    |
| Others                  | 14/303                      | 0.93 (0.40–2.13)      | 0.855    | 15/303                      | 0.78 (0.33–1.81)      | 0.558    | 18/390                        | 0.94 (0.37–2.43)       | 0.904    |
| Clinical stages         |                             |                       |          |                             |                       |          |                               |                       |          |
| I + II + IV             | 60/303                      | 1.29 (0.87–1.93)      | 0.211    | 63/303                      | 1.16 (0.78–1.74)      | 0.458    | 79/390                        | 1.39 (0.90–2.14)       | 0.135    |
| III + IV                | 61/303                      | 1.31 (0.87–1.96)      | 0.197    | 62/303                      | 1.26 (0.84–1.90)      | 0.259    | 77/390                        | 1.51 (0.98–2.31)       | 0.060    |

Note: *Adjusted for age and gender.

Abbreviations: CI, confidence interval; OR, odds ratio.
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 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* |
|----------------------------|---------------|------------------|----------|
|                            | n  | %    | n  | %    |          |
| Age range, months          |    |      |    |      |          |
| ≤ 18                       | 101 | 39.45 | 233 | 43.88 | 0.239    |
| > 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: *Two-sided \( \chi^2 \) test for distributions between neuroblastoma cases and controls.

Abbreviation: NA, not available.