The rs2147578 C > G polymorphism in the Inc-LAMC2–1:1 gene is associated with increased neuroblastoma risk in the Henan children

Tianyou Yang1, Zhuorong Zhang1, Jiao Zhang2, Tianbao Tan1, Jiliang Yang1, Jing Pan1, Chao Hu1, Jiahao Li1, Huimin Xia1, Jing He1* and Yan Zou1*

Abstract

Background: The rs2147578 C > G polymorphism in the long non-coding RNA gene Lnc-LAMC2–1:1 is associated with increased susceptibility to a few types of cancers. However, its role in neuroblastoma has not been evaluated yet.

Methods: We investigated the association between the lnc-LAMC2–1:1 rs2147578 C > G polymorphism and neuroblastoma susceptibility in Chinese Han populations. A total of 393 neuroblastoma cases and 812 healthy individuals from the Henan and Guangdong provinces were enrolled and subjected to genotyping. Odds ratio (OR) and 95% confidence interval (CI) were used to determine the strength of the association of interest.

Results: Combined analysis revealed that the lnc-LAMC2–1:1 rs2147578 C > G polymorphism was associated with increased neuroblastoma susceptibility (CG vs. CC: adjusted OR = 1.33, 95% CI = 1.01–1.75, P = 0.045; CG/GG vs. CC: adjusted OR = 1.34, 95% CI = 1.03–1.74, P = 0.028). In stratification analysis, children under 18 months with rs2147578 CG/GG genotypes had an increased neuroblastoma risk (adjusted OR = 1.70, 95% CI = 1.08–2.67, P = 0.022). Females with rs2147578 CG/GG genotypes also had increased neuroblastoma susceptibility (adjusted OR = 2.08, 95% CI = 1.37–3.18, P = 0.0007). In addition, children with lnc-LAMC2–1:1 rs2147578 CG/GG genotypes were prone to develop earlier stages of neuroblastoma (adjusted OR = 1.46, 95% CI = 1.01–2.12, P = 0.046).

Conclusions: The Lnc-LAMC2–1:1 rs2147578 C > G polymorphism may contribute to increased neuroblastoma susceptibility in children of Henan province.

Keywords: rs2147578, Neuroblastoma, Long non-coding RNA, Polymorphism

Background

Neuroblastoma is the most common malignant extracranial solid tumor in children, accounting for 7–10% of all tumors [1, 2]. Neuroblastoma originates from neural crest precursor cells of the sympathetic nervous system and is mainly located in the adrenal medulla, paraspinal ganglia, and sympathetic trunk [3–5]. The outcome of neuroblastoma is affected by several factors such as age of onset, pathological subtype, International Neuroblastoma Staging System (INSS) stage, N-myc status, DNA ploidy, and structural chromosomal aberrations [3–5].

Genetic factors are critically important in neuroblastoma tumorigenesis. Approximately 1% of the patients have a family history of neuroblastoma, and are carriers of certain genetic mutations. For instance, anaplastic lymphoma kinase (ALK) and PHOX2B gene variants are among the predisposing factors to familial neuroblastoma [6–8]. Evidence of genome-wide association studies (GWASs) of sporadic cases also suggests that genetic factors may be involved in the pathogenesis of neuroblastoma [9, 10]. These studies indicate an important role of genetic characteristics in the tumorigenesis of this disease.
Long non-coding RNAs (lncRNAs) are mRNA-like molecules whose genes belong to the non-protein coding genome. LncRNAs are involved in many biological processes such as gene imprinting, epigenetic regulation, translational regulation, splicing, and aging [11–15]. LncRNAs are also involved in apoptosis and cell differentiation, which are critical in tumorigenesis [16, 17]. LncRNAs such as HULC, PCAT-1, HOTAIR, ANRIL, and H19 are found to play important roles in cancer development [17]. Single nucleotide polymorphism (SNP) at HULC is associated with decreased hepatocellular carcinoma risk [18]. GWAS show that neuroblastoma patients with the G allele of SNP rs6939340, which is located in the lncRNA LOC729177 gene, have a high risk of metastasis and poor outcome [19, 20]. These studies indicate that evaluation of lncRNA gene polymorphism would be of great value in the risk assessment of neuroblastoma.

The rs2147578 C > G polymorphism in the lncRNA gene Lnc-LAMC2–1:1 is associated with susceptibility to several types of cancer, and functional polymorphism in Lnc-LAMC2–1:1 may confer a high risk of colorectal cancer through affecting miRNA binding [21]. Moreover, the Lnc-LAMC2–1:1 rs2147578 polymorphism is also considered a possible risk factor for acute lymphoblastic leukemia (ALL) in children [22]. However, few studies have focused on this polymorphism in neuroblastoma. Here we hypothesized that the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism may contribute to neuroblastoma susceptibility, and we tested our hypothesis via a case-control study.

Materials and methods

Study subjects

The subjects enrolled were described in previous studies [23, 24]. Briefly, 393 neuroblastoma patients and 812 cancer-free controls were enrolled from two different provinces of China: 275 neuroblastoma patients and 531 controls from the Guangzhou Women and Children’s Medical Center in Southern China, and 118 neuroblastoma patients and 281 controls from the Henan province in Northern China [25, 26]. Diagnosis and clinical stages of neuroblastoma were assigned according to the Shimada system and the international criteria for neuroblastoma staging system [27, 28]. Healthy controls had no history of malignancies and were matched to the neuroblastoma cases in terms of age (±5 years), gender, ethnicity, and geographical region. Both cases and controls were unrelated Chinese Han individuals living in the Guangdong and Henan provinces of China.

This study was approved by the Institutional Review Board of Guangzhou Women and Children’s Medical Center (Guangzhou, China), and written informed consent was obtained from the parents of the participants or their legal guardians for the use of their children’s medical data and biological samples.

SNP selection and genotyping

The Lnc-LAMC2–1:1 rs2147578 C > G polymorphism was genotyped using the TaqMan real-time PCR system on a 7900 Sequence Detection System (Applied Biosystems, Foster City, CA), as described previously [29–31]. Briefly, high-quality DNA samples were genotyped using Taqman real-time PCR method on a 7900 HT sequence detector system. The call rate for the SNPs was 99%, which met the pre-set criterion. For quality control, eight duplicate positive and eight negative controls without DNA were used in each 384-well plate [32, 33]. Additionally, 10% samples were randomly selected and repeated, and the reproducibility was 100% concordant.

Statistical analysis

All statistical tests were two-sided, with a significance level of P < 0.05. All statistical analyses were performed using SAS software (Version 9.4; SAS Institute, Cary, NC, USA). Two-sided χ² tests were used to analyze demographic data and genotype frequencies. The Hardy-Weinberg equilibrium was assessed using the goodness-of-fit χ² test. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated using the Woolf approximation method to evaluate association between the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism and neuroblastoma susceptibility. Crude and age- and gender-adjusted OR were evaluated using the unconditional logistic regression method.

Results

Demographic characteristics of the study population

A total of 275 cases of neuroblastoma and 531 health controls in Guangdong province and 118 neuroblastoma cases and 281 health controls in Henan province were evaluated (Additional File 1 Table S1). Age and gender distributions were similar between cases and controls in both Guangdong and Henan province subgroups (P > 0.05). Distribution of clinical stages and sites of tumor origin are also listed in Additional file 1 Table S1.

Lnc-LAMC2–1:1 rs2147578 C > G polymorphism and susceptibility to neuroblastoma

Genotype and allele frequencies of the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism and associations with neuroblastoma risk are summarized in Table 1. In both combined and subgroup analyses, the genotype distribution of the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism in the controls and cases were consistent with Hardy-Weinberg equilibrium (P = 0.164 for combined subjects, P = 0.279 for Guangdong province, and P = 0.386 for Henan province).
No significant difference in CC, CG, and GG genotype distributions was found in the Guangdong subgroup, indicating that the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism is not associated with neuroblastoma risk in the Guangdong study population. However, the lnc-LAMC2–1:1 rs2147578 C > G polymorphism was associated with increased neuroblastoma risk in the Henan population (CG vs. CC: adjusted OR = 1.73, 95% CI = 1.03–2.89, \( P = 0.048 \); CG/GG vs. CC: adjusted OR = 1.64, 95% CI = 1.00–2.68, \( P = 0.048 \)).

The combined analysis showed that the distribution of the CG genotype was significantly higher in the neuroblastoma group (adjusted OR = 1.33, 95% CI = 1.01–1.75, \( P = 0.045 \)), indicating that the Lnc-LAMC2–1:1 rs2147578 CG/GG genotype carriers had an increased risk of neuroblastoma (CG/GG vs. CC: adjusted OR = 1.34, 95% CI = 1.03–1.74, \( P = 0.028 \)).

### Stratification analysis of the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism and neuroblastoma risk

Stratification analyses according to age, gender, site of origin, and clinical stage were further conducted. The CG/GG genotypes in children younger than 18 months were associated with increased neuroblastoma risk (adjusted OR = 1.70, 95% CI = 1.08–2.67, \( P = 0.022 \)). Females with the CG/GG genotypes were associated with increased neuroblastoma risk (adjusted OR = 2.08, 95% CI = 1.37–3.18, \( P = 0.0007 \)). In addition, Individuals with the CG/GG genotypes tended to be in an earlier clinical stage of neuroblastoma (adjusted OR = 1.46, 95% CI = 1.01–2.12, \( P = 0.046 \)). Finally, no significant association between the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism and the site of tumor origin was found (Table 2).

### Discussion

In this study, we investigated the association between the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism and neuroblastoma susceptibility in Chinese Han populations. We found that the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism is associated with increased neuroblastoma susceptibility. Specifically, females and children younger than 18 months with specific genotypes in the Lnc-LAMC2–1:1 rs2147578 C > G polymorphism are at an increased risk of neuroblastoma. Fortunately,
individuals with the CG/GG variation tended to have the earlier stages of neuroblastoma.

Neuroblastoma accounts for approximately 15% of all childhood cancer mortality, and understanding the underlying mechanisms of this disease would be of great value for diagnosis and treatment [1, 2]. Genetic variants are critical lying mechanisms of this disease would be of great value for childhood cancer mortality, and understanding the under-

earlier stages of neuroblastoma.

LncRNA-MALAT1 and GAS5 being reported to mediate cell invasion, migration, and apoptosis in human neuroblastoma [34, 35]. The lnc-LAMC2−1:1 polymorphism is located in the LAMC1 gene near the LAMC2 gene [21]. Previous evidence also suggests that the lnc-LAMC2−1:1 rs2147578 C > G polymorphism may contribute to childhood ALL development [22]. Our results show that the lnc-LAMC2−1:1 rs2147578 C > G polymorphism may also be involved in neuroblastoma tumorigenesis.

Table 2 Stratification analysis of the association between the lnc-LAMC2−1:1 rs2147578 C > G polymorphism and neuroblastoma susceptibility for combined subjects

| Variable                   | rs2147578 (case/control) | Crude OR (95% CI) | P   | Adjusted OR * (95% CI) | P * |
|---------------------------|--------------------------|------------------|-----|------------------------|-----|
| Age, month                |                          |                  |     |                        |     |
| ≤ 18                      | 35/120                   | 1.70 (1.08–2.67) | 0.022 | 1.70 (1.08–2.67)       | 0.022 |
| > 18                      | 82/174                   | 1.18 (0.86–1.63) | 0.302 | 1.18 (0.86–1.62)       | 0.312 |
| Gender                    |                          |                  |     |                        |     |
| Female                    | 39/130                   | 2.04 (1.34–3.10) | 0.0009 | 2.08 (1.37–3.18)       | 0.0007 |
| Male                      | 78/164                   | 1.01 (0.73–1.42) | 0.938 | 1.00 (0.72–1.40)       | 0.981 |
| Site of origin            |                          |                  |     |                        |     |
| Adrenal gland             | 43/294                   | 1.46 (1.00–2.13) | 0.052 | 1.41 (0.96–2.07)       | 0.077 |
| Retroperitoneal region    | 31/204                   | 1.03 (0.65–1.63) | 0.903 | 1.04 (0.66–1.66)       | 0.863 |
| Mediastinum               | 32/294                   | 1.37 (0.89–2.12) | 0.157 | 1.39 (0.90–2.16)       | 0.138 |
| Others                    | 9/294                    | 1.71 (0.79–3.68) | 0.171 | 1.75 (0.81–3.78)       | 0.155 |
| Clinical stage            |                          |                  |     |                        |     |
| I + II + 4 s              | 46/294                   | 1.44 (0.99–2.08) | 0.055 | 1.46 (1.01–2.12)       | 0.046 |
| III + IV                  | 68/294                   | 1.20 (0.87–1.65) | 0.272 | 1.16 (0.84–1.61)       | 0.362 |

* Adjusted for age and gender

OR odds ratio; CI confidence interval

The values were in bold if the 95% CI excluded 1, or P<0.05

LAMC2 can interact with the epidermal growth factor receptor (EGFR), and influence its downstream pathway [38]. Previous studies revealed that the EGF receptor is overexpressed in neuroblastoma tissues and cells, and anti-EGFR agents are potential targeted therapies for neuroblastoma [39–41]. The possible interaction between the lnc-LAMC2–1:1 rs2147578 polymorphism and the EGFR pathway may account for the increased risk of neuroblastoma of the G allele.

Our results from Guangdong (Southern China) and Henan (Northern China) provinces were inconsistent. In the Henan province subgroup, the CG genotype distribution was significantly higher in the neuroblastoma group, and subjects with the GG and CG genotypes had a significantly increased risk of neuroblastoma. In contrast, no association between the G allele and neuroblastoma was found in the Guangdong province subgroup. A possible explanation for this inconsistency may be the relatively complex genetic background of the Guangdong Chinese Han population. Studies on Y-chromosome phylogeny suggest that people in Southern China, including Guangdong province, are much more polymorphic than populations in Northern China, including Henan province [42–44]. However, the relatively small sample size of our study may introduce bias.

Conclusion

The lnc-LAMC2–1:1 rs2147578 C > G polymorphism is associated with increased neuroblastoma susceptibility in Han populations of Northern China. Female individuals and children younger than 18 months with such genetic variants are
at an increased risk for neuroblastoma. But with samples collected from only two provinces, we can’t make any solid conclusion. We might look into this question in the near future when we collect more samples.

Additional file

Additional file 1: Table S1. Clinical characteristics of neuroblastoma cases and cancer-free controls. (DOCX 15 kb)

Abbreviations

ALK: Anaplastic lymphoma kinase; ALL: Acute lymphoblastic leukemia; CI: Confidence interval; EGRF: Epidermal growth factor receptor; GWAS: Genome-wide association study; INSS: International Neuroblastoma Staging System; LncRNA: Long non-coding RNAs; OR: Odds ratio; SNP: Single nucleotide polymorphism

Funding

This study was funded by the Pearl River S&T Nova Programme of Guangzhou (No: 201710010086), the National Natural Science Foundation of China (No: 81602199), the Guangzhou Science Technology and Innovation Commission (No: 201607010395), the Natural Science Foundation of Guangdong Province, China (No: 2016A030313406), and National Natural Science Foundation of China (No: 81602199). The funding body has no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

TY, JH, YZ made substantial contributions to conception and design of this study. ZZ, JZ, TT, JP, CH, and JL make substantial contribution to the acquisition of data, and interpretation of data. TY, JH, JY, and HX made substantial contribution to statistical analysis and interpretation of data. TY, SL, YZ had been involved in drafting the manuscript and revising it critically for important intellectual content. YZ and JH were agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. TY, and ZZ contributed to the work equally. All authors had given final approval of the version to be published.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Guangzhou Women and Children’s Medical Center (Guangzhou, China). Written informed consent was obtained from the parents of the participants or their legal guardians for the use of their children’s medical data and biological samples. All patient records/data were anonymized and de-identified prior to analysis.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Department of Pediatric Surgery, Guangzhou Women and Children’s Medical Center, Guangzhou Medical University, 9 Jinshi Road, Guangzhou 510623, Guangdong, China. 2Department of Pediatric Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China.
26. Zhang J, Zhuo ZJ, Wang J, He J, Yang L, Zhang D, et al. CASC15 gene polymorphisms reduce neuroblastoma risk in Chinese children. Oncotarget. 2017;8(19):34344–9.

27. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi W, Roald B, et al. The international neuroblastoma pathology classification (the Shimada system). Cancer. 1999;86:364–72.

28. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol. 1993;11:1466–77.

29. Gong J, Tian J, Lou J, Wang X, Ke J, Li J, et al. A polymorphic MYC response element in NBTD1 influences colorectal cancer risk, especially in interaction with a MYC regulated SNP rs6983267. Ann Oncol. 2018;29:632–9.

30. Li J, Zou L, Zhou Y, Li L, Zhu Y, Yang Y, et al. A low-frequency variant in SMAD7 modulates TGF-beta signaling and confers risk for colorectal cancer in Chinese population. Mol Carcinog. 2017;56:1798–807.

31. Lou J, Gong J, Ke J, Tian J, Zhang Y, Li J, et al. A functional polymorphism located at transcription factor binding sites, rs6695837 near LAMC1 gene, confers risk of colorectal cancer in Chinese populations. Carcinogenesis. 2017;38:177–83.

32. He J, Qiu LX, Wang MY, Hua RX, Zhang RX, Yu HP, et al. Polymorphisms in the XPG gene and risk of gastric cancer in Chinese populations. Hum Genet. 2012;131:1235–44.

33. He J, Wang F, Zhu J, Zhang R, Yang T, Zou Y, et al. Association of potentially functional variants in the XPG gene with neuroblastoma risk in a Chinese population. J Cell Mol Med. 2016;20(1):481–90.

34. Bi S, Wang C, Li Y, Zhang W, Zhang J, Lv Z, et al. LncRNA-MALAT1-mediated Axl promotes cell invasion and migration in human neuroblastoma. Tumour Biol. 2017;39:1010428317699796.

35. Mazar J, Rosado A, Shelley J, Marchica J, Westmoreland TJ. The long non-coding RNA GAS5 differentially regulates cell cycle arrest and apoptosis through activation of BRCA1 and p53 in human neuroblastoma. Oncotarget. 2017;8(38):6589–6607.

36. Takahashi S, Hasebe T, Oda T, Sasaki S, Kinoshita T, Konishi M, et al. Cytoplasmic expression of laminin gamma2 chain correlates with postoperative hepatic metastasis and poor prognosis in patients with pancreatic ductal adenocarcinoma. Cancer. 2002;94:1894–901.

37. Yamamoto H, Itoh F, Iku S, Hosokawa M, Imai K. Expression of the gamma(2) chain of laminin-5 at the invasive front is associated with recurrence and poor prognosis in human esophageal squamous cell carcinoma. Clin Cancer Res. 2001;7:896–900.

38. Garg M, Kanodia D, Okamoto R, Jain S, Madan V, Chien W, et al. Laminin-5 gamma-2 (LAMC2) is highly expressed in anaplastic thyroid carcinoma and is associated with tumor progression, migration, and invasion by modulating signaling of EGFR. J Clin Endocrinol Metab. 2014;99(6):E62–72.

39. Evangelopoulos ME, Weis J, Kruttschnitt A. Signalling pathways leading to neuroblastoma differentiation after serum withdrawal: HDL blocks neuroblastoma differentiation by inhibition of EGFR. Oncogene. 2005;24:3309–18.

40. Michaels M, Bliss J, Arnold SC, Hirsch N, Rothweiler F, Deubzer HE, et al. Cisplatin-resistant neuroblastoma cells express enhanced levels of epidermal growth factor receptor (EGFR) and are sensitive to treatment with EGFR-specific toxins. Clin Cancer Res. 2008;14:6531–7.

41. Zheng C, Shen R, Li K, Zheng N, Zong Y, Ye D, et al. Epidermal growth factor receptor is overexpressed in neuroblastoma tissues and cells. Acta Biochim Biophys Sin. 2016;48:762–7.

42. Deng W, Shi B, He X, Zhang Z, Xu J, Li B, et al. Evolution and migration history of the Chinese population inferred from Chinese Y-chromosome evidence. J Hum Genet. 2004;49:339–48.

43. Shen P, Wang F, Underhill PA, Franco C, Yang WH, Roxas A, et al. Population genetic implications from sequence variation in four Y chromosome genes. Proc Natl Acad Sci U S A. 2000;97:7354–9.

44. Underhill PA, Shen P, Lin AA, Jin L, Passarino G, Yang WH, et al. Y chromosome sequence variation and the history of human populations. Nat Genet. 2000;26:358–61.