CELL CYCLE
2020, VOL. 19, NO. 18, 2367–2372
https://doi.org/10.1080/15384101.2020.1808382

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RESEARCH PAPER

lncRNA-uc003opf.1 rs11752942 A>G polymorphism decreases neuroblastoma risk in Chinese children

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ABSTRACT

Recent studies have revealed that long non-coding RNAs (lncRNAs) play critical roles in the tumorigenesis and proliferation of human cancer. Several polymorphisms of lncRNAs have been found to be involved in the risk of neuroblastoma (NB). However, studies on the relationship between polymorphisms in lncRNA exons and NB are infrequent. We evaluated the association between rs11752942 A > G polymorphism in *lnc-RNA-uc003opf.1* exon and neuroblastoma susceptibility by performing a hospital-based study with 275 patients and 531 controls. Odds ratios (ORs) and 95% confidence intervals (CIs) assessed by using logistic regression models were used to determine the strength of the association. We found that the rs11752942 G allele is significantly associated with decreased neuroblastoma risk (AG vs. AA: adjusted OR = 0.72, 95% CI = 0.53–0.98, $P = 0.038$; and AG/GG vs. AA: adjusted OR = 0.74, 95% CI = 0.55–0.99, $P = 0.045$) after adjusting for age and gender. This association was more prominent in females, subjects with tumor in the mediastinum or early-stage. Furthermore, the expression quantitative trait locus analysis indicated that rs11752942 G was associated with decreased expression of its neighboring gene *LRFN2* mRNA. These results indicate that *lncRNA-uc003opf.1* may be a novel potentially functional lncRNA that may be used as a predictive marker, for it might contribute to decreased neuroblastoma risk.

ARTICLE HISTORY
Received 6 January 2020
Revised 8 July 2020
Accepted 6 August 2020

KEYWORDS
LncRNA-uc003opf.1; neuroblastoma; susceptibility

Introduction

Neuroblastoma (NB) is one of the most common malignancies among young and infant patients, and is the third leading cause of cancer-related death in children [1]. NB accounts for 8–10% of all childhood cancers and affects more than 10 million children globally [2]. This disease is the most common cancer that occurs in the first year of life, with a median age of diagnosis of 17 months [3]. Approximately, 65% of these tumors present in the abdomen, but they can also occur in the neck, pelvis and chest [4]. As an embryonal malignancy, the exact cause of NB remains unclear. Genome-wide association studies (GWASs) provide new methods for research on carcinogenesis and served as a powerful tool to enhance our understanding of cancer genetics [5]. Several common genetic variants or susceptibility loci for NB have been identified by GWASs, such as *BARD1, TP53, HACE1*, and *LIN28B* [6–8].

GWASs greatly expand our knowledge of disease phenotype-related SNPs, and note that 88% of disease-related SNPs are located in non-coding region [9]. Long noncoding RNAs (lncRNAs) are defined as transcribed RNA molecules that are >200 nucleotides, lacking open reading frame, and having no obvious protein-coding capacity [10]. Although the exact biological functions of most lncRNAs have not yet been determined, new evidences indicated that lncRNAs served as the major drivers of carcinogenesis and an important part of tumor biology [11]. Polymorphisms and aberrant expression of lncRNAs are associated with the susceptibility of multiple malignances [12], including NB. For example, *HOTAIR* rs920778 TT carriers could increase esophageal

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The supplemental data for this article can be accessed here.

squamous cell carcinoma (ESCC) risk in Chinese population, compared with the CC carriers [13]. LncRNA *H19* SNPs may contribute to susceptibility to gastric cancer [14]. *HOTAIR* rs920778 TT carriers had a significant increased gastric cancer risk in Chinese populations compared with the CC carriers [15]. *PCAT1* rs7463708 increases binding of ONECUT2, a novel androgen receptor (AR)-interacting transcription factor and promotes prostate cancer cell proliferation and tumor growth [16]. Moreover, the risk variants of *PCAT1* rs11672691 could also promote prostate cancer tumor growth and metastasis by decreasing the binding of transcription factors NKX3.1 and YY1 [17]. Taken together, these findings reveal that lncRNA SNPs mediated various mechanisms to impact the risk of different cancers. Several polymorphisms of lncRNAs have been found to be involved in the risk of NB, including *H19, LINC00673*, and *HOTAIR* [18–20]. Studies on the relationship between polymorphisms in lncRNA exons and NB are infrequent. Variant genotype of rs11752942 in *lnc-RNA-uc003opf.1* exon have been reported to be associated with the cancer risk. The rs11752942 A > G polymorphism could affect cell proliferation and tumor growth thereby infect the susceptibility of ESCC [21]. However, the association between potentially functional *lncRNAuc003opf.1* rs11752942 A > G polymorphism and NB susceptibility has not been reported. Herein, we performed a hospital-based case-control study using data from 275 neuroblastoma patients and 531 control subjects to evaluate the association between the rs11752942 A > G polymorphism and neuroblastoma risk in Southern Chinese children.

Materials and methods

Study subjects

As shown in Supplemental Table 1, 275 patients with newly diagnosed and histopathological confirmed neuroblastoma were enrolled in the study [22–24]. In addition, 531 age- and gender-matched healthy controls were randomly selected from children undergoing physical examination during the same period. At the time of recruitment, demographic factors and medical histories were collected using a structured questionnaire. Written informed consent was obtained from the participants' parents or legal guardians. The research protocol was approved by the Institutional Review Board of the Guangzhou Women and Children's Medical Center.

Polymorphism selection and analysis

The *lncRNA-uc003opf.1* rs11752942 A > G polymorphism was genotyped using the Taqman real-time PCR method as described previously [25]. Additionally, 10% of the samples were repeated randomly, and the results were 100% concordant.

Statistical analysis

A goodness-of-fit χ^2 test was performed to test for deviations from the Hardy-Weinberg equilibrium in terms of genotype frequencies of the polymorphism in control individuals. A two-sided χ^2 test was used to evaluate differences in demographic variables and frequency distributions of genotypes between patients and controls. We conducted unconditional univariate logistic regression to estimate the association between the *lncRNA-uc003opf.1* rs11752942A>G polymorphism and neuroblastoma susceptibility by computing the odds ratios (ORs) and 95% confidence intervals (CIs). Adjusted ORs were calculated by performing multivariate analysis, adjusting for age and gender. Expression quantitative trait loci (eQTL) analysis in the GTEx portal (https://www.gtexportal.org/home/) was adopted to determine the correlation between the SNP rs11752942A>G and level of its neighboring genes expression [26]. All statistical analyzes were performed using SAS software. $P < 0.05$ was considered statistically significant.

Results

The *lncRNA-uc003opf.1 rs11752942 A > G* polymorphism is associated with decreased neuroblastoma risk

The characteristics of the 275 patients with neuroblastoma and 531 controls included in the current study were described previously [22–24]. Of them, 275 patients and 525 controls were successfully

genotyped. The observed genotype frequency was in accordance with the Hardy-Weinberg equilibrium in control subjects ($P = 0.375$). Genotype frequencies of *lncRNA-uc003opf.1* rs11752942A>G in cases and controls are presented in Table 1. We found that carriers of the rs11752942 G allele was significantly associated with a decreased risk of NB after adjusting for age and gender (AG vs. AA: adjusted OR = 0.72, 95% CI = 0.53–0.98, $P = 0.038$; and AG/GG vs. AA: adjusted OR = 0.74, 95% CI = 0.55–0.99, $P = 0.045$).

Stratification analysis

We performed subgroup analyzes by age, gender, tumor site of origin, and clinical stage to evaluate the effects of the rs11752942 A > G polymorphism on neuroblastoma risk (Table 2). We found that the rs11752942 AG/GG genotypes were significantly associated with a decreased risk of neuroblastoma in females (adjusted OR = 0.60, 95% CI = 0.38–0.94, $P = 0.026$), subjects with tumors in the mediastinum (adjusted OR = 0.49, 95% CI = 0.31–0.77, $P = 0.002$) and patients with early-stage tumors (adjusted OR = 0.52, 95% CI = 0.35–0.79, $P = 0.002$).

Effect of rs11752942 A > G on the expression of neighboring genes

We further assessed the functional relevance of rs11752942 A > G using released data from GTEx. The rs11752942 G allele was significantly associated with lower expression level of its neighboring gene *leucine-rich repeat and fibronectin type III domain-containing protein 2* (*LRFN2*) in the whole blood (Figure 1).

Discussion

In the present hospital-based case-control study with 275 neuroblastoma patients and 531 controls, we investigated the relationship between *lncRNA-uc003opf.1* rs11752942 polymorphism and neuroblastoma risk. We observed a significant association between the rs11752942 A > G polymorphism and neuroblastoma susceptibility among Southern Chinese children. In addition, stratified analysis showed that rs11752942 G allele carriers in the following subgroup were less likely to suffer NB: females, subjects with tumors in the mediastinum, and patients with tumors of early clinical stages.

LncRNAs are defined as autonomously transcribed non-coding RNAs longer than 200 nucleotides that do not overlap annotated coding genes [27]. Over the past decade, studies have confirmed that long non-coding RNAs (lncRNAs) are involved in various cellular functions, such as transcriptional and translational regulation, as well as variance in gene expression. Accumulating evidence has demonstrated that genetic variation of lncRNA may alter the structure, affect the stability and influence the expression level of lncRNA, and contribute to carcinogenesis [10]. Large intergenic non-coding RNAs (lincRNA), a subset of lncRNAs, are expressed from a genomic locus between the protein-coding loci [28]. The importance of lincRNA genes are showed by their characteristics, such as a tendency for locations next to developmental regulators, enrichment of tissue-specific expression patterns, high conservation among species, and frequent association with genetic traits [29]. Many functional SNPs of lincRNA have been demonstrated to be involved in initiation and progression of different types of malignancies, acting as either a tumor suppressor or

Table 1. Genotype distributions of *lncRNA-uc003opf.1* rs11752942 A > G polymorphism and neuroblastoma susceptibility.

Genotype	Cases (n = 275)	Controls (n = 525)	P [a]	Crude OR (95% CI)	P	Adjusted OR (95% CI) [b]	P [b]
rs11752942 A > G (HWE = 0.375)							
AA	131 (47.64)	211 (40.19)		1.00		1.00	
AG	112 (40.73)	251 (47.81)		**0.72 (0.53–0.98)**	**0.038**	**0.72 (0.53–0.98)**	**0.038**
GG	32 (11.64)	63 (12.00)		0.82 (0.51–1.32)	0.411	0.82 (0.51–1.33)	0.426
Additive			0.113	0.84 (0.67–1.05)	0.119	0.84 (0.68–1.05)	0.124
Dominant	144 (52.36)	314 (59.81)	0.043	**0.74 (0.55–0.99)**	**0.043**	**0.74 (0.55–0.99)**	**0.045**
Recessive	243 (88.36)	462 (88.00)	0.880	0.97 (0.61–1.52)	0.881	0.97 (0.62–1.53)	0.901

OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.
Results were in bold, if the 95% CI excluded 1 or $\boldsymbol{P} < \mathbf{0}.05$.
[a]χ^2 test for genotype distributions between neuroblastoma cases and cancer-free controls.
[b]Adjusted for age and gender.

Table 2. Stratification analysis for the association between *lncRNA-uc003opf.1* rs11752942 A > G polymorphism and neuroblastoma susceptibility.

	rs11752942 (cases/controls)					
Variables	AA	AG/GG	Crude OR (95% CI)	*P*	Adjusted OR [a] (95% CI)	*P* [a]
Age, month						
≤18	48/89	55/141	0.72 (0.45–1.16)	0.176	0.73 (0.45–1.16)	0.180
>18	83/122	89/173	0.76 (0.52–1.10)	0.148	0.76 (0.52–1.11)	0.151
Gender						
Females	56/84	58/146	0.60 (0.38–0.94)	0.026	0.60 (0.38–0.94)	0.026
Males	75/127	86/168	0.87 (0.59–1.28)	0.468	0.86 (0.58–1.26)	0.437
Sites of origin						
Adrenal gland	27/211	37/314	0.92 (0.54–1.56)	0.759	0.93 (0.55–1.57)	0.776
Retroperitoneal	37/211	50/314	0.91 (0.57–1.44)	0.681	0.92 (0.58–1.46)	0.734
Mediastinum	52/211	38/314	0.49 (0.31–0.77)	0.002	0.49 (0.31–0.77)	0.002
Others	13/211	13/314	0.67 (0.31–1.48)	0.323	0.68 (0.31–1.50)	0.338
Clinical stages						
I+ II+4s	65/211	51/314	0.53 (0.35–0.79)	0.002	0.52 (0.35–0.79)	0.002
III+IV	61/211	82/314	0.90 (0.62–1.31)	0.595	0.90 (0.62–1.31)	0.580

OR, odds ratio; CI, confidence interval.
Results were in bold, if the 95% CI excluded 1 or $P < 0.05$.
[a]Adjusted for age and gender, omitting the corresponding stratify factor.

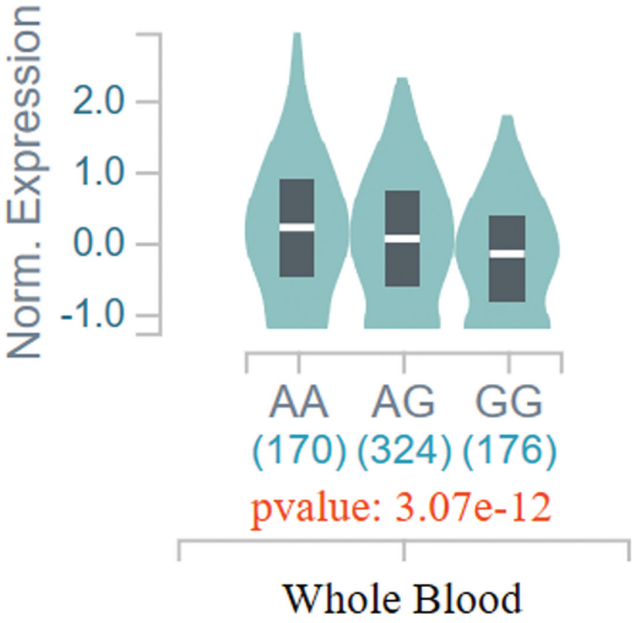

Figure 1. Functional relevance of rs11752942 A > G on its neighboring gene expression in GTEx database. rs11752942 G genotype had significantly lower *LRFN2* mRNA level in the whole blood, when compared to rs11752942 A genotype ($P = 3.07*10^{-12}$).

promoter, which can be turned into new diagnosis markers and therapeutic targets [30].

The *lncRNA-uc003opf.1* at 6p21.2 is actually a lincRNA. *lncRNA-uc003opf.1* rs11752942A>G polymorphism was first studied by Wu et al. who conducted a case-control study with 1493 cases and 1553 controls to explore its association with ESCC risk [21]. They reported that when compared with the A allele, the rs11752942 G allele could markedly attenuate the level of lincRNA-uc003opf.1 by binding miRNA-149. Further functional experiments suggested that rs11752942 G genotype could contribute to G2 phase arrest, so that cells could not smoothly pass through G2/M to split. This suggests that *lncRNA-uc003opf.1* plays an important role in cell cycle and thus interferes with cell proliferation and tumor growth, which finally impact risk of ESCC. This demonstrated that SNP has potential functional effects on lncRNA levels and susceptibility to cancer [30]. In current hospital case-control studies, we have also found that rs11752942 AG and AG/GG genotypes reduce the risk of neuroblastoma in Chinese groups. We further attempted to interpret the possible mechanism of rs11752942 A > G-mediated neuroblastoma risk. eQTL evidence suggested that the G allele in rs11752942 is significantly associated with decreased *leucine-rich repeat and fibronectin type III domain-containing protein 2* (*LRFN2*) level in the whole blood. The protein of LRFN2, as a component of membrane, has been found to interact with N-methyl-D-aspartate receptors (NMDARs) to participate in the neuron development and synapse function [31]. Furthermore, several studies indicated that NMDARs played important roles in the development and progression of multiple cancers, including gastric cancer, colorectal carcinoma and ovarian cancer [32–34]. However, the exact functional roles of

rs11752942 A > G and its neighboring gene *LRFN2* in neuroblastoma are unknown. Therefore, further studies are worthwhile to validate whether this SNP could affect the expression of *lncRNA-uc003opf.1* in neuroblastoma or not.

This study demonstrated a significant association between *lncRNA-uc003opf.1* rs11752942 A > G polymorphism and neuroblastoma risk in Chinese children. However, several limitations should be addressed. First, the sample size in the current study was not large enough because of the very low incidence of neuroblastoma. The relatively small sample size might result in limited statistical power. Second, selection bias might exist, because all subjects were enrolled only in our hospital and restricted to a Chinese population. Thus, we might possibly have omitted a large number of neuroblastoma cases in patients who did not visit our hospital for treatment during the same period. Therefore, our study population might not be representative of the general Chinese population. Third, as a result of the nature of the retrospective study design, some selection and information bias might be inevitable. These biases could only be reduced by frequency-matching cases and controlling for age and sex, to some extent, as information on paternal exposures, dietary intake, and living environment was not available. Fourth, here we only assess the impact of genetic variants on neuroblastoma risk. Whether and how rs11752942 A > G influence the growth of neuroblastoma cells is not carried out in the current stage. More functional experiments are needed to explore possible function and mechanism of rs11752942 A > G in neuroblastoma. At any rate, *lncRNA-uc003opf.1* may be a novel potentially functional lincRNA that may be used as a predictive marker, for it involved in susceptibility to NB.

In conclusion, we revealed that the *lncRNA-uc003opf.1* rs11752942 A > G polymorphism may take part in neuroblastoma susceptibility in Southern Chinese children, and might be a candidate for biological marker. Nevertheless, further well-designed prospective studies with larger sample sizes of different ethnicities, as well as further functional studies, are needed to validate and confirm our findings.

Authors' contributions

All authors contributed significantly to this work. J.P., T.-Y. Y., J.-L. Y., C.H., J.-H. Z., and T.-B. T. performed the research study and collected the data; J.H. and J.-H. L. analyzed the data; J.P., T.-Y. Y., J.H., H.-M. X., and Y. Z. designed the research study; J.P., H.-R.L., T.-Y. Y., J.H., and Y.Z. wrote the paper, and J.H. prepared all the Tables. All authors reviewed the manuscript. In addition, all authors approved the final draft.

Disclosure statement

The authors confirm that there are no conflicts of interest.

Funding

This study was funded by the Pearl River S&T Nova Program of Guangzhou (No: 201710010086), the National Natural Science Foundation of China (No: 81602199), the Guangzhou Science Technology and Innovation Commission (No: 201607010395), and the Natural Science Foundation of Guangdong Province, China (No: 2016A030313496).

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