Journal of Inflammation Research

Dovepress
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ORIGINAL RESEARCH

Single-Nucleotide Polymorphism LncRNA AC008392.1/rs7248320 in CARD8 is Associated with Kawasaki Disease Susceptibility in the Han Chinese Population

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Background: Kawasaki disease (KD) is a multisystem vasculitis in infants and young children and involved in the NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome activation. Genetic factors may increase the risk of KD. To assess the association between rs7248320 in long noncoding RNA (lncRNA) AC008392.1 located in the upstream region of CARD8 and the risk of KD, a case–control study was conducted in the Han Chinese population.

Methods: This study genotyped the polymorphism rs7248320 in the lncRNA AC008392.1 gene using the TaqMan real-time polymerase chain reaction assay. The genetic contribution of rs7248320 was evaluated using odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression analysis. The association between rs7248320 and KD susceptibility was analyzed by performing a hospital-based case–control study including 559 KD patients and 1055 non-KD controls.

Results: In this study, a significant relationship between rs7248320 and KD risk was observed in the genotype/allele frequency distribution. The rs7248320 polymorphism was associated with a significantly decreased risk of KD after adjustment for age and sex (AG vs AA: adjusted OR = 0.80, 95% CI: 0.64–0.99, $P = 0.0421$; GG vs AA: adjusted OR = 0.71, 95% CI: 0.51–1.00, $P = 0.0492$; AG/GG vs AA: adjusted OR = 0.78, 95% CI: 0.63–0.96, $P = 0.0186$). Moreover, the rs7248320 G allele also exhibited a decreased risk for KD (adjusted OR = 0.83, 95% CI: 0.72–0.97, $P = 0.0193$) compared with the A allele. In the stratification analysis, compared to the rs7248320 AA genotype, AG/GG genotypes were more protective for males (OR = 0.71, 95% CI: 0.55–0.93, $P = 0.0122$).

Conclusion: This study suggests for the first time that the lncRNA AC008392.1 rs7248320 polymorphism may be involved in KD susceptibility in the Han Chinese population.

Keywords: Kawasaki disease, rs7248320, AC008392.1, CARD8, lncRNA, single-nucleotide polymorphism

Introduction

Kawasaki disease (KD) usually occurs in infants and young children and can lead to the occurrence of coronary aneurysms in approximately 25%[1] of untreated cases.[2,3] There is still a risk of arterial endothelial dysfunction in adolescent and adult patients, even those without coronary artery lesions (CALs) in the acute phase of KD.[4,5] Currently, the incidence of KD is gradually increasing, especially that of incomplete KD, which leads to earlier diagnosis and more complication.[6]

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Received: 29 July 2021
Accepted: 9 September 2021
Published: 21 September 2021

Many studies have indicated that KD is involved in aspects of autoimmune diseases.[7–9] For example, autoimmunity-associated thrombosis initiated by the binding of anti-endothelial cell autoantibodies to endothelial cells might play an essential role in the pathogenesis of certain subtypes of KD.[10] Innate and adaptive immune responses presenting dysregulated conditions are also evident in the acute stages of KD. More importantly, the NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome is a key driver of KD vasculitis.[11,12] Anzai et al found that NLRP3 inflammasome is crucial for the development of vasculitis in a KD murine model induced by *Candida albicans* water-soluble fraction.[13] The endothelial cell pyroptosis may play an important role in coronary endothelial damage of KD by activating NLRP3 inflammasome.[14] In summary, inflammation is considered to be responsible for the development of KD, which is archetypal pediatric vasculitis, exemplifying the unique aspects and challenges of vascular inflammation in the pediatric patient population.[15] NLRP3 inflammasome activation plays a crucial role in the immunopathogenesis of KD.

Currently, although KD pathogenesis is still undetermined, epidemiological and clinical evidence suggests that KD is also associated with inter-individual variability in genetic susceptibility to KD and differences in the prevalence of KD among ethnicities are related to genes.[16] The genes that are studied in KD can be classified into 4 major groups: enhanced T cell activation, decreased apoptosis, dysregulated B cells and altered TGF-β signaling.[17] In the first KD genome-wide association studies (GWAS), some gene polymorphisms (such as CSMD1, lnx1, ZFHX3, CAMK2D, and tcp1) were suggested to be associated with the risk of KD.[18] Additionally, single-nucleotide polymorphisms (SNPs) in rs7922552, rs2833195, rs4786091, rs17076896, rs2254546, rs4813003, rs12068753, rs16921209, rs2857151 and some SNPs in CD40, FCGR2A and BLK may be related to KD susceptibility, clinical manifestations (such as CAL), treatment, laboratory diagnostic indicators (such as C-reactive protein) and sex in KD based on previously presented data.[19–25] However, the etiology of KD remains unclear. A large fraction of hereditary factors may have a potential impact on the development of genetic diagnosis and prognosis for KD.

Caspase-associated recruitment domain (CARD) 8, as a member of the family of CARD proteins, which are conserved homology domain and protein–protein interaction modules,[26] a negative regulator of NLRP3 inflammasome,[27,28] which is a cytoplasmic protein complex that plays an essential role in promoting the maturation and secretion of pro-inflammatory cytokines.[29–31] These cytokines were shown to be strongly implicated in inflammation and autoimmune processes by inducing the adaptive immune response.[29,30,32] Moreover, some studies linked to the gene polymorphisms of CARD8 are also important in the pathogenesis of diseases related to inflammation and immune function, such as atopic dermatitis,[33] rheumatoid arthritis,[34,35] Crohn's disease,[36] gout,[37] and ankylosing spondylitis.[38] Accordingly, genetic variants of CARD8 with immunosuppressive activity may be related to the risk of KD.

Long non-coding RNAs (LncRNAs) can play the roles of regulators of protein-coding genes.[39] Generally, lncRNAs are essential parts of "dark matter" and are not translated into proteins.[40,41] Nevertheless, emerging evidence highlights the mechanistic role of lncRNAs in diverse biological and physio-pathological contexts, such as in immune responses, neuronal disorders and cancer,[42] and lncRNAs can influence pathological progression.[43,44] LncRNA AC008392.1 located in the upstream region of CARD8 in the long arm of the 9-chromosome. The expression of lncRNA AC008392.1 is found in many human cell lines, such as B cells and tumor cells.[45,46] The study has shown that expression quantitative trait loci (eQTL) for CARD8 may be represented by rs7248320 in AC008392.1, which may affect CARD8 expression.[46] According to Yin et al's study, the variant rs7248320GG genotype contributes to risks of hepatocellular carcinoma and cervical cancer.[46] Therefore, we hypothesized that the rs7248320 SNP may alter KD susceptibility by affecting CARD8 expression. However, to date, there have been no studies on the association between the rs7248320 gene polymorphism and KD susceptibility or CAL complications; that is, the relation between AC008392.1/rs7248320 and the etiopathogenesis of KD is unknown. In this study, to assess the role of rs7248320 in lncRNA AC008392.1 in KD susceptibility, we conducted current case–control study based on a hospital from which 559 cases and 1055 non-KD controls were selected in the Han Chinese population.

Materials and Methods

Study Population

According to the Declaration of Helsinki, the study was supported by the Ethics Committee of Beijing Children's Hospital, Capital Medical University, and all participants signed an informed consent form (ID: 2019-4). In this study,

all KD cases and non-KD controls with unrelated kinship were from the Han Chinese population. A total of 559 patients with KD were recruited mainly from the Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, China between February 2019 and December 2020. All of the KD cases were classified mainly according to the guidelines for diagnosis, treatment, and long-term management of KD prescribed by the American Heart Association in 2017.[1] The inclusion criteria for KD cases were as follows: 1) patients with confirmed KD, 2) no treatment, and 3) no previous cancer or metastatic cancer.

Additionally, 1055 non-KD controls were recruited in accordance with the inclusion criteria: 1) without a family history of KD, 2) without history of cancer or metastatic cancer, 3) without autoimmune conditions, 4) selected from our hospital during the same period, 5) fever for more than 5 days, and 6) matched to case subjects by age and sex.

DNA Extraction and SNP Genotyping

SNPs were selected using the NCBI dbSNP database and SNP information in this study. SNP LncRNA AC008392.1/rs7248320 specification is shown in Table 1 (https://www.ncbi.nlm.nih.gov/snp/rs7248320?vertical_tab=true).

Genomic DNA was extracted from a total of 0.2 mL blood with EDTA within one week from each subject (Tiangen, Beijing, China). Then, high-quality genomic DNA samples were used for genotyping through the TaqMan real-time polymerase chain reaction assay on an ABI Q6 instrument (Thermo Fisher Scientific, Tempe, Arizona, United States). Specific primers were purchased from Thermo Fisher Scientific (Tempe, Arizona, United States). Ten percent of the samples were also randomly selected for repeated measurements, and the results showed that the concordance rate was 100%.

Statistical Analysis

The genotype distribution of SNP rs7248320 and the demographic variables between KD patients and non-KD controls were compared using the χ^2 test. Hardy–Weinberg equilibrium (HWE) in controls was tested using the goodness-of-fit χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) calculated by unconditional logistic regression were used to analyze the association between the AC008392.1/rs7248320 SNP and KD susceptibility. Stratified analysis was implemented for age, sex and coronary artery outcomes. Data analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). All significance tests in this study were two-sided, and P value <0.05 was considered statistically significant. Additionally, continuous variables are displayed as the means ± standard deviations and ranges (min, max). Categorical variables are described as frequencies and/or percentages.

Table 1 SNP LncRNA AC008392.1/rs7248320 Reference Report

SNP	Gene	Location (GRCh38.p13)	Polymorphism	Gene Feature
rs7248320	CARD8	Chr.19: 48256972	A/G	2KB Upstream Variant

Results

Baseline Characteristics

The baseline characteristics of the KD patients and non-KD controls are summarized in Table 2. The mean ages were 33.18 ± 28.56 (1.00–192.00) months for KD patients and 35.74 ± 32.16 (1.00–168.00) months for controls. There were no significant differences in the distribution of age (P = 0.1157) and sex (P = 0.1306) between KD cases and non-KD controls. According to two-dimensional echocardiography, 143 (25.58%) patients developed CALs, and 406 (72.63%) developed non-coronary artery aneurysms (NCALs).

Table 2 Characteristics in KD Cases and Non-KD Controls

Variables	Cases (n, %)		Controls (n, %)		*P*-value[a]
Total	559	100%	1055	100%	
Age, months	33.18 ± 28.56 (1.00–192.00)		35.74 ± 32.16 (1.00–168.00)		
≤60 months	494	88.37%	903	85.59%	0.1157
>60 months	65	11.63%	152	14.41%	
Sex					
Male	360	64.40%	639	60.57%	0.1306
Female	199	35.60%	416	39.43%	
Severity of coronary artery lesions					
Unknown[b]	10	1.79%	0	0.00%	
CALs[c]	143	25.58%	0	0.00%	
NCALs[d]	406	72.63%	1055	100%	

Notes: [a]Two-sided χ^2 test for distributions between Kawasaki disease cases and non-Kawasaki disease controls. [b]Kawasaki disease patients with unknown coronary artery status. [c]Kawasaki disease patients with coronary artery lesions. [d]Kawasaki disease patients without coronary artery lesions.

Association Between the AC008392.1/rs7248320 Polymorphism and KD Susceptibility

Frequency distribution of the rs7248320 SNP polymorphism in the KD patients and non-KD controls is shown in Table 3. The rs7248320 gene was in accordance with HWE in the controls (P = 0.1214). A statistically significant association of the rs7248320 polymorphism (P = 0.0493) and a decreased the risk of KD after adjustments for age and sex (AG vs AA: adjusted OR = 0.80, 95% CI: 0.64–0.99, P = 0.0421; GG vs AA: adjusted OR = 0.71, 95% CI: 0.51–1.00, P = 0.0492; AG/GG vs AA: adjusted OR = 0.78, 95% CI: 0.63–0.96, P = 0.0186) was observed but not GG vs AG/AA: adjusted OR=0.81, 95% CI: 0.59–1.11, P = 0.1820. Nevertheless, as a result, the rs7248320 G allele genotype showed a decreased risk for KD (adjusted OR = 0.83, 95% CI: 0.72–0.97, P = 0.0193) compared with the A allele genotype in the overall distribution.

Stratified Analysis of the AC008392.1/rs7248320 Polymorphism and KD Susceptibility

This study further explored the associations of AC008392.1/rs7248320 gene polymorphism with the risk of KD in the stratified analysis of sex, age and CAL formation (Table 4). Compared with the rs7248320 AA genotype, the variant AG/GG genotypes exhibited more protection for males (OR = 0.71, 95% CI: 0.55–0.93, P = 0.0122) but were not significantly associated with the children aged ≤60 or >60 months (adjusted P > 0.05). The variant AG/GG

Table 3 Genotype Frequency Distribution of the AC008392.1/rs7248320 Polymorphism in KD Cases and Non-KD Controls

Genotype/Allele	Cases (n, %)	Controls (n, %)	*P*-value[a]	OR (95% CI)	*P*-value	Adjusted OR (95% CI)	Adjusted *P*-value[b]
rs7248320 (HWE=0.1214)							
AA	242 (43.29)	393 (37.25)	**0.0493***	1.00		1.00	
AG	254 (45.44)	518 (49.10)		0.80 (0.64–0.99)	**0.0420***	0.80 (0.64–0.99)	**0.0421***
GG	63 (11.27)	144 (13.65)		0.71 (0.51–0.99)	**0.0466***	0.71 (0.51–1.00)	**0.0492***
Dominant model	317 (56.71)	662 (62.75)	**0.0184***	0.78 (0.63–0.96)	**0.0182***	0.78 (0.63–0.96)	**0.0186***
Recessive model	496 (88.73)	911 (86.35)	0.1700	0.80 (0.59–1.10)	0.1744	0.81 (0.59–1.11)	0.1820
A	738 (66.01)	1304 (61.80)	**0.0179***	1.00		1.00	
G	380 (33.99)	806 (38.20)		0.83 (0.72–0.97)	**0.0183***	0.83 (0.72–0.97)	**0.0193***

Notes: [a]Two-sided χ^2 test for distributions between Kawasaki disease cases and non-Kawasaki disease controls. [b]Adjusted for sex and age status in logistic regression models. *Bold values: The statistically significant (*P*-values < 0.05).

Table 4 Stratification Analysis of the AC008392.1/rs7248320 Polymorphism in KD Cases and Non-KD Controls

Variables	rs7248320 (Cases/Controls)		*P*-value[a]	OR (95% CI)	*P*-value	Adjusted OR (95% CI)	Adjusted *P*-value[b]
	AA	AG/GG					
Age, months							
≤60	215/349	279/554	0.0764	0.82 (0.65–1.02)	0.0761	0.81 (0.65–1.01)	0.0652
>60	27/44	38/108	0.0731	0.57 (0.31–1.05)	0.0716	0.62 (0.34–1.16)	0.1332
Sex							
Male	155/219	205/420	**0.0061***	0.69 (0.53–0.90)	**0.0060***	0.71 (0.55–0.93)	**0.0122***
Female	87/174	112/242	0.6572	0.93 (0.66–1.30)	0.6570	0.93 (0.66–1.31)	0.6735
Severity of coronary artery lesions							
CALs[c]	65/393	78/662	0.0604	0.71 (0.50–1.01)	0.0590	0.71 (0.49–1.01)	0.0549
NCALs[d]	173/393	233/662	0.0604	0.80 (0.63–1.01)	0.0598	0.81 (0.64–1.02)	0.0674

Notes: [a]Two-sided χ^2 test for distributions between Kawasaki disease cases and non-Kawasaki disease controls. [b]Adjusted for sex and age status in logistic regression models. [c]Kawasaki disease cases with coronary artery lesions. [d]Kawasaki disease cases without coronary artery lesions. *Bold values: The statistically significant (*P*-values < 0.05).

genotypes were also not significantly associated with the risk of CAL formation (adjusted $P > 0.05$) compared with the variant AA genotypes.

Discussion

To date, although numerous studies on KD have been performed, the etiology of KD remains unknown. Generally, KD is not a genetic disease. However, evidence indicates that there are genetic components associated with the risk of KD.[1] GWAS and family linkage studies have also been implicated KD susceptibility and SNPs, which represent the most common type of genetic variation within the population.[1,47] Genetic tests play important roles in the prediction of cancer risk, drug resistance, outcome, prognosis, and susceptibility to environmental factors, while SNP variations hold great potential as biomarkers.[48,49] In the KD, a substantial polygenic component is still not completely clear but would have an impact on the development of genetic diagnosis and prognosis.

LncRNAs are potential biomarkers in numerous human diseases and provide a theoretical basis for clinically targeting them.[38,39] LncRNAs regulate gene expression in various ways and play significant roles in cellular processes, such as differentiation,[50] growth,[51] apoptosis,[52] proliferation, migration and invasion,[53] metastasis and autophagy.[54] Currently, there is a lack of research on the association between the gene polymorphisms of lncRNA AC008392.1 in CARD8 and the occurrence of KD. CARD8 is an important component of NLRP3 inflammasome that has played an immunosuppressive role by inhibiting NLRP3 inflammasome activation,[27,28] which is a key driver of KD vasculitis.[11–14] To our knowledge, this is the first study on the KD susceptibility and the lncRNA AC008392.1/rs7248320 polymorphism, which may affect the normal expression of CARD8.[46,55]

In this case–control study, we examined the relationship between the KD susceptibility and the CARD8 lncRNA AC008392.1/rs7248320 polymorphism in 559 KD patients and 1055 non-KD controls. The results revealed that the rs7248320 G allele was associated with decreased KD susceptibility in the Han Chinese population. Furthermore, the rs7248320 G variant demonstrated a protective effect on the occurrence of KD for male children by stratified analysis. Therefore, the rs7248320 SNP and somatic mutation in lncRNA AC008392.1 may have strong and great potential as biomarkers in the pathogenesis of KD with further development of lncRNAs.

Studies have reported that KD leads to more apparent effects in young children aged <60 months; 9 to 11 months of age is the peak incidence of onset; and the male to female incidence is 1.5 to 1.[56,57] However, compared to the AC008392.1/rs7248320 AA genotype, the protective effect of the variant AG/GG genotypes was not demonstrated to be more predominant in children below 60 months of age in our study. The lncRNA AC008392.1/rs7248320 A > G polymorphism might not affect the risk of KD in terms of age, excluding other interferential conditions, such as an uncharacteristic Chinese population that was biased toward children in northern China in the current study.

Additionally, KD is relevant to systemic vasculitis and particularly affects the coronary arteries, causing major CAL complications.[1–3] However, the factors imposing a high risk on the formation of CAL coronary complications are still not completely clear. Several candidate genes have previously been proposed to be involved in CAL formation in KD, such as TBXA2R rs4523,[58] inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) rs2290692[59] and CD40 rs4810485.[60] However, in our study, stratified analysis results showed that the CARD8 rs7248320 G variant was not a protective factor against the formation of KD CALs. There could be an explanation that the sample size of this study may be too small to obtain a statistically significant result involving in age and CAL complications. Further studies of the association between the AC008392.1/rs7248320 polymorphism and KD are necessary.

Currently, there are also many other studies on CARD8 polymorphisms for their activity under different conditions. For example, the CARD8 rs2043211 polymorphism was shown to protect against noise-induced hearing loss;[61] the CARD8 rs2043211 polymorphism may be associated with gout;[37] the CARD8 rs10403848 polymorphism was significantly associated with psoriasis vulgaris,[62] as the CARD8 rs7248320 polymorphism influenced KD susceptibility in our study. More SNP genes of CARD8 associated with KD susceptibility, treatment, prognosis, and outcome need to be clarified in the future.

Limitations

This study has several limitations. First, this study is a hospital-based case–control study, which might result in Berkson's bias, a selection bias in the distribution of disease amongst hospitalized patients. Second, only genetic factors, but not disease history, medication history and environmental factors, etc., were taken into account. Third, larger sample-size studies are needed to

further confirm the association between the rs7248320 SNP and the KD susceptibility. Finally, there is a lack of experiments to determine the association between the rs7248320 genotypes and the expressions of lncRNA AC008392.1 and CARD8. Nevertheless, a previous study showed that the lncRNA AC008392.1 rs7248320 polymorphism may represent an eQTL for CARD8.[46] It has been reported that the genotypes of eQTL SNPs are associated with the expression of the corresponding lncRNA.[63] We will try to address these issues based on current results in the future.

Conclusions

In conclusion, this study confirmed that the lncRNA AC008392.1/rs7248320 G allele in the CARD8 gene was associated with decreased occurrence of KD and provided a protective effect in decreasing KD susceptibility for male children. Nevertheless, further studies with larger sample sizes and practical experiments should be performed to further explore the roles of the SNP rs7248320 in the CARD8 gene in terms of the risk of KD and CAL complications.

Data Sharing Statement

Please contact the author for data requests.

Ethical Approval

The studies involving human participants were reviewed and approved by the Ethics Committee of Beijing Children's Hospital, Capital Medical University (Project number: 2019-4). Written Informed consents were obtained from each participant's guardian.

Acknowledgments

The authors would like to thank the Department of Clinical Laboratory Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, China for providing the clinical samples. We thank our colleagues from the Department of Clinical Biological Resource Bank, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, China for their technical assistance.

Author Contributions

All authors contributed significantly to this work. All authors made substantial contributions to the conception and design of the study as well as the acquisition, analysis and interpretation of data. All authors participated in drafting the article or revising it critically for important intellectual content, and all authors agreed to submit the manuscript to the current journal. All authors gave final approval for the version to be published and agreed to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors report no conflicts of interest in this work.

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