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ORIGINAL RESEARCH

The Associated of the Risk of IVIG Resistance in Kawasaki Disease with *ZNF112* Gene and *ZNF180* Gene in a Southern Chinese Population

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Background: Kawasaki disease (KD) was one of the most common primary vasculitis. IVIG resistance was associated with an increased risk of coronary artery aneurysm. Accumulating evidences demonstrated that inflammatory gene polymorphisms might play important roles in IVIG resistance, and zinc finger proteins were closely related to immune inflammation regulation, but the effect of *ZNF112*/rs8113807 and *ZNF180*/rs2571051 on IVIG resistance in KD patients has not been reported.

Methods: A total of 996 KD patients were recruited, and the assay of TaqMan-real-time polymerase chain reaction was used for *ZNF112*/rs8113807 and *ZNF180*/rs2571051 genotyping. Odds ratio (OR) and 95% confidence interval (CI) were calculated for estimating the relationship between the polymorphisms of the both SNPs (*ZNF112*/rs8113807 and *ZNF180*/rs2571051) and the risk of IVIG resistance.

Results: Both of the *ZNF112*/rs8113807 CC/TC genotype and the *ZNF180*/rs2571051 TT/CT genotype increased the risk of IVIG resistance in KD (rs8113807: CC vs TT: adjusted OR = 1.83, 95% CI = 1.06–3.16, p = 0.0293; CC/TC vs TT adjusted: OR = 1.49, 95% CI = 1.10–2.02, p = 0.0094. rs2571051: TT vs CC adjusted: OR = 2.64, 95% CI = 1.62–4.29, p < 0.0001; TT/CT vs CC adjusted: OR = 2.14, 95% CI = 1.37–3.37, p = 0.0009; TT vs CC/CT adjusted: OR = 1.66, 95% CI = 1.22–2.27, p = 0.0014). Furthermore, the combinative analysis of risk genotypes in *ZNF112*/rs8113807 and *ZNF180*/rs2571051 showed that patients with two unfavorable genotypes were more likely to increase risk of IVIG resistance than those who carried with zero or one unfavorable genotypes (adjusted: OR = 1.68, 95% CI = 1.24–2.27, p = 0.0008).

Conclusion: Our findings enriched the genetic background of IVIG resistance risk in the KD development and suggested that the *ZNF112*/rs8113807 C-carrier and the *ZNF180*/rs2571051 T-carrier were associated with increased risk of IVIG resistance in KD patients in Chinese southern population.

Keywords: Kawasaki disease, *ZNF112*/rs8113807, *ZNF180*/rs2571051, IVIG resistance, inflammatory gene, polymorphism

Introduction

Kawasaki disease (KD) was an acute, self-limiting vasculitis mainly in infants and young children[1–3] and due to the unknown etiology of KD, this posed a great challenge to clinical treatment. Failure to receive timely IVIG treatment and IVIG resistance (persistent or recrudescent fever at least 36 hours and <7 days after completion of first IVIG infusion[4]) in clinical practice were associated with increased risk of life-threatening complications.[5] Such as vascular stenosis, arterial thrombosis, coronary artery disease[6,7] and Kawasaki shock syndromes or KD-Macrophage activation syndrome

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leading to death.[8,9] Research found that the incidence of IVIG resistance in children was increasing, with approximately 10–20% of patients not responding to IVIG. In recent years, studies have shown that IVIG resistance in KD patients might be related to genetic factors, among which *HMGB1* and *SAMD9L* gene polymorphisms were related to IVIG resistance.[10,11] Studies have reported that the level of IL-6 in KD patients' serum was related to IVIG resistance, and KD patients with IVIG resistance were treated with anti-TNF -α or anti-IL-1 therapy (for their inflammation and immune response).[12–14] Therefore, we speculated that some genes regulating inflammatory response might be related to IVIG resistance.

Zinc finger protein and related genes were demonstrated to play an important role in cardiovascular disease and inflammatory response. Okada et al have shown that Zinc finger transcript factor Early Growth Response gene-1 mediates inflammation from damage to blood vessels and thrombosis (KD was also vasculitis).[15] Other studies have confirmed that *ZNF174* affects the course of vascular injury through PDGF-B.[16,17] Cattaruzza et al demonstrated the inhibitory effect of zinc finger motif-1 (*ZFM1*) on proinflammatory gene expression in vascular smooth muscle cells.[18] Zinc finger protein 180 (*ZNF180*), also known as *HHZ168*, was a protein-coding gene that acts as a molecular regulator of immune cell infiltration in melanoma cells, enhancing CD8+T cell toxicity and negatively correlated with PAI-1 expression.[19] Therefore, *ZNF180* might also modulate immune cell infiltration by affecting PAI-1. At present, there were few studies on *ZNF112*, but many of them have shown that the ZNF family was related to inflammation, and *ZNF112* (member of ZNF family) might also be involved in inflammation. In addition, studies have also shown that ZNF-related regions were associated with IVIG resistance.[20] The genetic susceptibility of *ZNF112* and *ZNF180* gene polymorphisms in IVIG resistance in KD patients has not been studied. Therefore, in this study, we focused on whether *ZNF112*/ rs8113807 T>C and *ZNF180*/rs2571051 C>T were related to IVIG resistance in KD patients from a southern Chinese population.

Materials and Methods
Study Population
A total of 996 KD patients were recruited by Department of Pediatric Cardiology of Guangzhou Women and Children's Medical Center. The diagnosis of KD was based on guidelines issued by the American Heart Association;[1,21] IVIG resistance inclusion criteria accorded that persistent or recrudescent fever at least 36 hours and less than 7 days after completion of first IVIG infusion.[4,5] All parents of patients signed the informed consents. The research followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (2014073009, 2018052702, 2021093A01).

DNA Extraction and SNP Genotype
We selected two SNPs of *ZNF112*/rs8113807and *ZNF180*/2571051. Total genomic DNA was isolated from 200μL blood samples using TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the reagent company's instructions. Genotyping was carried on by real-time fluorescence quantitative PCR (QuantStudio™ 6 Flex Real-Time PCR System, Thermo Fisher Scientific, CA, USA). Later, the 10% of the samples (randomly selected) were repeated genotyping, and the results were consistent to the first genotyping.

Statistical Analysis
All the statistical analysis was performed on the SAS software (version 9.4, SAS Institute, Cary, NC, United States). The differences of genotype frequencies distributions between IVIG resistance and IVIG sensitivity in the KD patients were assessed via using two-sided χ^2 test. The strength of the association between rs8113807/rs2571051 polymorphisms and IVIG resistance in the KD progression was assessed by using unconditional multivariate logistic regression for calculating odds ratio (OR) and 95% confidence interval (CI), including adjustment of age and gender. Via the same statistical method, the further analysis of age/gender stratification was carried out to explore the relationship of the two SNPs' polymorphisms and IVIG resistance in the KD progression. P-value less than 0.05 was considered statistically significant.

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Analysis of Expression Quantitative Trait Loci (eQTL) and Splicing Quantitative Trait Loci (sQTL)

The expression quantitative trait loci (eQTL) and splicing quantitative trait loci (sQTL) of rs8113807 and rs2571051 were analyzed by genotype-tissue expression (GTEx) (http://www.gtexportal.org/).

Results

Characteristics of KD Patients

The characteristics of patients are presented in Table 1. In this study, 996 KD patients were recruited from Guangzhou Women and Children's Medical Center and divided into IVIG resistance group (KD-IVIG resistance, 219 patients) and IVIG sensitivity group (KD-IVIG sensitivity, 777 patients). The age range of the KD-IVIG resistance group was 2–132 months (Mean ± SD = 25.09± 21.33), while the KD-IVIG sensitivity group was 1–131 months (Mean ± SD = 25.79± 20.61). There was no significant difference in the distributions of age (p = 0.7633) and gender (p = 0.1658) between the two groups.

Association Between Selected SNP and IVIG Resistance Susceptibility in KD Patients

The genotype frequencies distributions of *ZNF112*/rs8113807 and *ZNF180*/rs2571051 in KD patients are shown in Table 2. We found that the CC/TC genotype of *ZNF112*/rs8113807 was a risk factor for IVIG resistance in KD patients (CC vs TT adjusted: OR = 1.83, 95% CI = 1.06–3.16, p = 0.0293; Dominant model: CC/TC vs TT adjusted: OR = 1.49, 95% CI = 1.10–2.02, p = 0.0094) and *ZNF180*/rs2571051 TT/CT genotype was a dangerous factor for IVIG resistance in KD patients (TT vs CC adjusted: OR = 2.64, 95% CI = 1.62–4.29, p = <0.0001; Dominant model: TT/TC vs CC adjusted: OR = 2.14, 95% CI = 1.37–3.37, p = 0.0009; Recessive model: CC/TC vs TT adjusted: OR = 1.66, 95% CI = 1.22–2.27, p = 0.0014). In the current study, via combining effect of the two risk genotypes of *ZNF112*/rs8113807 and *ZNF180*/rs2571051 genotypes, we found that there was a higher risk of IVIG resistance in patients carrying two genotypes rather than those carrying 0–1 genotypes (adjusted: OR = 1.68, 95% CI = 1.24–2.27, p = 0.0008).

Stratification Analysis

We conducted a stratified analysis of the risk of IVIG resistance for the two SNPs in KD patients by age and gender. The results are shown in Table 3. The results indicated that both age and gender were associated with IVIG resistance in KD patients. Compared to *ZNF112*/rs8113807 TT genotypes, a significantly increasing risk of IVIG

Table 1 Characteristics in Kawasaki Disease Patients with IVIG Resistance and Sensitive Therapy

Variables	KD-IVIG Resistance		KD-IVIG Sensitivity		p^a
	NO.	%	NO.	%	
Total	219		777		
Age range, month	2–132		1 −131		
Mean ± SD	25.09 ± 21.33		25.79 ± 20.61		
≤60	206	94.06	735	94.59	0.7633
>60	13	5.94	42	5.41	
Gender					
Male	160	73.06	530	68.21	0.1658
Female	59	26.94	247	31.79	

Note: ^aTwo-sided χ2 test for distributions between Kawasaki disease patients with IVIG resistance and sensitive therapy.

Table 2 Genotype Frequency Distribution of Polymorphisms in *ZNF112*/rs8113807 and *ZNF180*/rs2571051 Between KD Cases with IVIG Resistance and Sensitive Therapy

Genotype	IVIG Resistance (n=219, %)	IVIG Sensitivity (n=777, %)	*P*-value[a]	OR (95% CI)	*P*-value[a]	Adjusted OR (95% CI)	Adjusted *P*-value[b]
ZNF112/rs8113807							
TT	99 (45.21)	426 (54.83)	**0.0299**	1.00		1.00	
TC	98 (44.75)	298 (38.35)		1.42 (1.03–1.94)	**0.0313**	1.44 (1.04–1.97)	**0.0260**
CC	22 (10.05)	53 (6.82)		1.79 (1.04–3.08)	**0.0362**	1.83 (1.06–3.16)	**0.0293**
Additive				1.37 (1.09–1.72)	**0.0081**	1.39 (1.10–1.75)	**0.0061**
Dominant	120 (54.79)	351 (45.17)	**0.0118**	1.47 (1.09–1.99)	**0.0120**	1.49 (1.10–2.02)	**0.0094**
Recessive	197 (89.95)	724 (93.18)	0.1219	1.53 (0.91–2.57)	0.1121	1.55 (0.92–2.62)	0.0987
ZNF180/rs2571051							
CC	25 (11.42)	169 {21.75}	**0.0002**	1.00		1.00	
CT	105 (47.95)	382 (49.16)		1.86 (1.16–2.98)	**0.0101**	1.85 (1.15–2.97)	**0.0112**
TT	89 (40.64)	226 (29.09)		2.66 (1.64–4.33)	**<0.0001**	2.64 (1.62–4.29)	**<0.0001**
Additive				1.58 (1.26–1.97)	**<0.0001**	1.57 (1.26–1.96)	**<0.0001**
Dominant	194 (88.58)	608 (78.25)	**0.0003**	2.16 (1.38–3.38)	**0.0008**	2.14 (1.37–3.37)	**0.0009**
Recessive	130 (59.36)	551 (70.91)	**0.0014**	1.67 (1.22–2.28)	**0.0012**	1.66 (1.22–2.27)	**0.0014**
Combined effect of risk genotypes[c]							
0	14 (6.39)	108 (13.90)	**0.0003**	1.00		1.00	
I	96 (43.84)	379 (48.78)		1.95 (1.07–3.56)	**0.0287**	1.95 (1.07–3.56)	**0.0292**
2	109 (49.77)	290 (37.32)		2.90 (1.59–5.28)	**0.0005**	2.91 (1.60–5.31)	**0.0005**
Trend				1.60 (1.26–2.03)	**0.0001**	1.61 (1.27–2.04)	**<0.0001**
0–I	110 (50.23)	487 (62.68)	**0.0010**	1.00		1.00	
2	109 (49.77)	290 (37.32)		1.66 (1.23–2.25)	**0.0010**	1.68 (1.24–2.27)	**0.0008**

Notes: [a]Two-sided χ^2 test for distributions between Kawasaki disease patients with IVIG resistance and sensitive therapy. [b]Adjusted for gender and age status in logistic regression models. [c]Risk effect combine analysis (rs8113807 TC/CC + rs2571051 TT/CT) in logistic regression models. Statistically significant values are shown in bold (P < 0.05).

resistance in KD patients carried with *ZNF112*/rs8113807 TC/CC genotypes was observed in children younger than 60 months of age (adjusted: OR = 1.48, 95% CI = 1.80–2.02, p = 0.0146) or female (adjusted: OR = 2.73, 95% CI = 1.47–5.07, p = 0.0015); Meanwhile, *ZNF180*/rs2571051 TT/CT genotypes increased the risk of IVIG resistance compared with CC genotypes in KD patients who were younger than 60 months of age (adjusted: OR = 2.13, 95% CI = 1.35–3.38, p = 0.0013) or male (adjusted: OR = 2.16, 95% CI = 1.25–3.72, p = 0.0057). Furthermore, the combined analysis of the two risk genotypes (*ZNF112*/rs8113807 TC/CC + *ZNF180/*rs2571051 TT/CT) indicated that two risk genotypes apparently increased IVIG resistance in patients younger than 60 months of age (adjusted: OR = 1.65, 95% CI = 1.21–2.25, p = 0.0017) and female (adjusted: OR = 2.83, 95% CI = 1.57–5.10, p = 0.0005).

Analysis of eQTL and sQTL

We further used GTEx database to analyze the eQTLs and sQTLs of rs2571051/rs8113807. As shown in Figure 1, rs2571051 polymorphisms was significantly associated with *ZNF*180 mRNA expression, meanwhile the TT genotype showed higher expression in Muscle-skeletal (P = 1.40×10^{-11}) (Figure 1A), Thyroid (P = 1.6×10^{-5}) (Figure 1B), Brain-Cerebellar Hemisphere (P = 2.3×10^{-5}) (Figure 1C), and Brain-Frontal Cortex (BA9) (P = 3.6×10^{-5}) (Figure 1D) and influenced *ZNF180* splicing events in Cells-Cultured fibroblasts (P = 1.70×10^{-9}) (Figure 1E), Vagina (P = 3.10×10^{-7}) (Figure 1F), Artery-Tibial (P = 4.1×10^{-6}) (Figure 1G), and Adipose-Subcutaneous (P = 5.4×10^{-6}) (Figure 1H). We did not find any messages related to rs811380 (eQTLs or sQTLs) in GTEx database, and the relationship between rs8113807 and the expression of *ZNF112* needs to be further explored.

Table 3 Stratification Analysis of Polymorphisms in *ZNF112*/rs8113807 and *ZNF180*/rs2571051 Between KD Cases with IVIG Resistance and Sensitive Therapy

Variables	*ZNF112*/rs8113807 (IVIG Resistance/IVIG Sensitivity)		Adjusted OR (95% CI)	Adjust P-value[a]	*ZNF180*/rs2571051 (IVIG Resistance/IVIG Sensitivity)		Adjusted OR (95% CI)	Adjust P-value[a]	Combined Analysis[b] (IVIG Resistance/IVIG Sensitivity)		Adjusted OR (95% CI)	Adjust P-value[a]
	TT	**TC/CC**			**CC**	**TT/CT**			**0–1**	**2**		
Age, months												
≤60	92/396	114/339	1.48 (1.08–2.02)	**0.0146**	24/163	182/572	2.13 (1.35–3.38)	**0.0013**	103/456	103/279	1.65 (1.21–2.25)	**0.0017**
>60	7/30	6/12	2.17 (0.59–7.91)	0.2419	1/6	12/36	2.00 (0.22–18.32)	0.5403	7/31	6/11	2.48 (0.66–9.24)	0.1768
Gender												
Male	82/297	78/233	1.21 (0.85–1.73)	0.2915	17/108	143/422	2.16 (1.25–3.72)	**0.0057**	88/332	72/198	1.37 (0.96–1.96)	0.0849
Female	17/129	42/118	2.73 (1.47–5.07)	**0.0015**	8/61	51/186	2.12 (0.95–4.73)	0.0667	22/155	37/92	2.83 (1.57–5.10)	**0.0005**

Notes: [a]Adjusted for gender and age status in logistic regression models. [b]Risk effect combine analysis (rs8113807 TC/CC + rs2571051 TT/CT) in logistic regression models. Statistically significant values are shown in bold (P < 0.05).
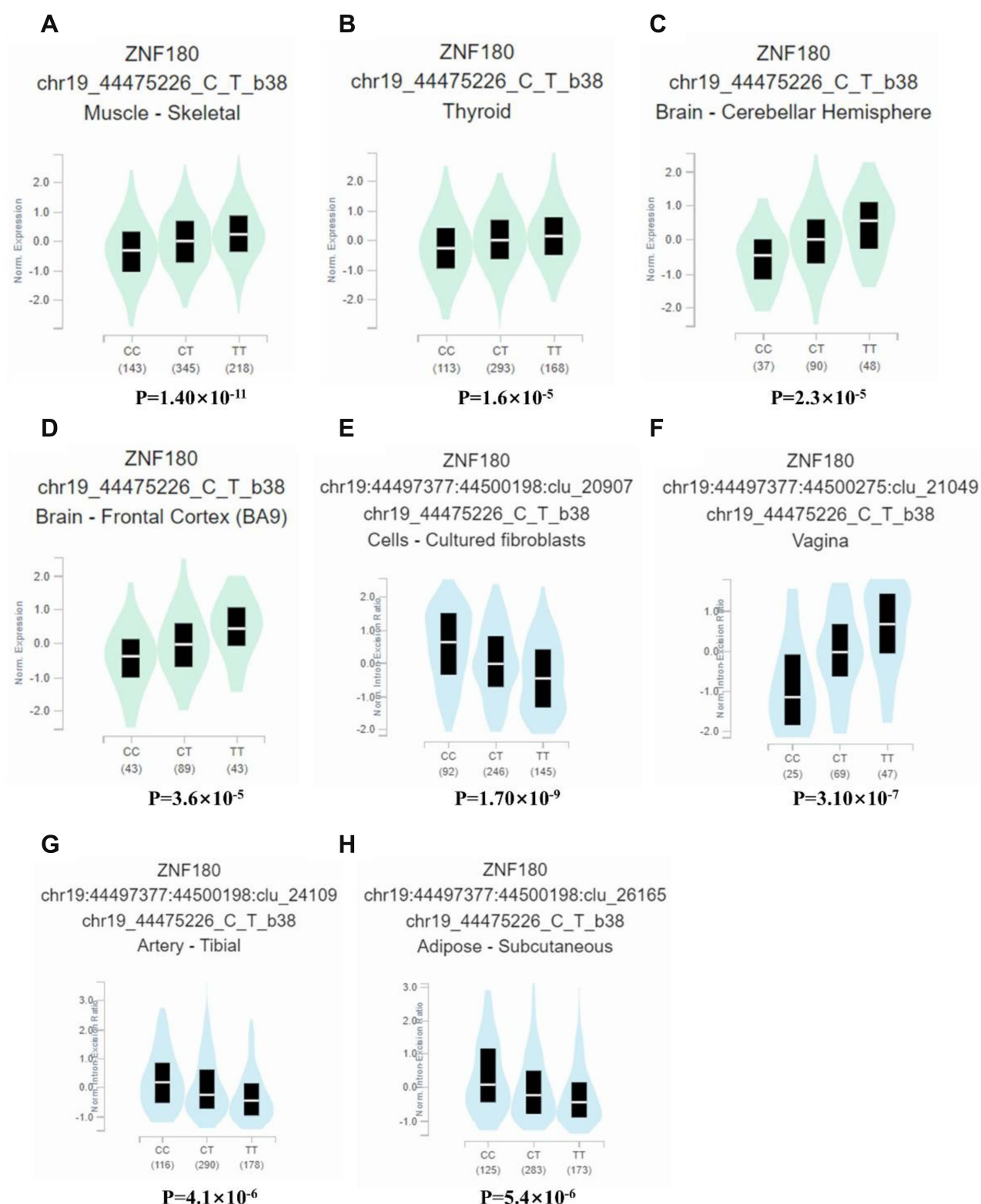
Figure 1 Functional relevance of rs2571051 on gene expression and splicing events in GTEx database. rs2571051 was significantly associated with *ZNF180* level in the (**A**) Muscle-skeletal (P = 1.40×10⁻¹¹), (**B**) Thyroid (P = 1.6×10⁻⁵), (**C**) Brain-Cerebellar Hemisphere (P = 2.3×10⁻⁵) and (**D**) Brain-Frontal Cortex (BA9) (P = 3.6×10⁻⁵); rs2571051 can affect the splicing events of (**E**) Cells-Cultured fibroblasts (P = 1.70×10⁻⁹), (**F**) Vagina (P = 3.10×10⁻⁷), (**G**) Artery-Tibial (P = 4.1×10⁻⁶) and (**H**) Adipose-Subcutaneous (P = 5.4×10⁻⁶).

Discussion

Previous studies have indicated that 10–20% of KD patients did not respond to initial IVIG therapy, and these patients were at higher risk of developing CALs or CAA.[5] The cause of non-response to IVIG therapy and the mechanism of action of IVIG therapy were still unknown. Therefore, it is important to elucidate the mechanisms to prevent the development of CALs or CAA. In this study of 219 cases in the IVIG resistance group and 777 cases in the IVIG sensitivity group, we revealed that *ZNF112*/rs8113807 TC/CC genotype and *ZNF180*/rs2571051 TT/CT genotype were associated with the incidence of IVIG resistance in KD patients. *ZNF112*/rs8113807 TC/CC genotype significantly increased the susceptibility of KD to IVIG resistance, and also the TT/CT genotype of *ZNF180*/rs2571051 was a dangerous factor of KD patients with IVIG resistance. These findings further assessed the risk of IVIG resistance in KD patients from the level of genetic variation, which might help to elucidate the mechanisms or risk factors of IVIG non-response and have the opportunity to predict the risk of IVIG resistance in KD patients.

KD was an acute vascular inflammation, and its development causes inflammatory response and the release of pro-inflammatory factors. IVIG treatment was a commonly used treatment method, and the changes in clinical patients treated with IVIG explain more about the anti-inflammatory effect. For example, Franco et al[22] reported that the number of Treg cells in peripheral blood of KD patients in convalescence was increased after initial IVIG injection but was not found in the coronary arteries of patients who did not respond to IVIG therapy. This suggested that Treg regulation might play a role in the mechanism of IVIG action on KD; Shrestha et al 's[23] newly finding indicated that the *NA1* variant of FcγRIIB was associated with lack of response to IVIG. Studies have shown that the initial IVIG resistant patients with increasing risk of coronary artery anomalies occur.[24,25] As a transcription factor that played a role in both inflammation and anticoagulation, many studies have confirmed that the ZNF family played an important role in the transcription regulation of various cardiovascular diseases and inflammatory responses in physiological or pathological processes. Weiss et al found that *ZNF263* was a transcription inhibitor of heparin and HS-specific biosynthetic enzymes (*HS3ST1* and *HS3ST3A1*).[26] Zinc finger motif-1 (*ZFM1*) inhibits pro-inflammatory gene expression in vascular smooth muscle cells (SMC)[18] and zinc finger protein Miz1 limited hepatocellular driven inflammation to inhibit Hepatocellular carcinoma (HCC).[27] Lv et al[28] demonstrated that zinc finger protein 191 (*ZFP191*) played a key role in proliferation and migration of aortic vascular smooth muscle cells (VSMC) through animal modeling. Some studies have confirmed that *ZEB1* was associated with migration and invasion of hemangioma cells and that gene polymorphisms in the *ZEB2* region were associated with the risk of coronary artery disease.[29,30] According to the current studies on *ZNF*180, studies have shown the potential immune regulation effect and promotes the proliferation of tumor cells.[19] In summary, the ZNF family was associated with cardiovascular diseases, *ZNF180* was associated with inflammatory immune regulation, and IVIG treatment affects the immune response of KD patients. Although there are just few studies on *ZNF112*, there was a potential possibility that *ZNF112* was associated with the occurrence of IVIG resistance. Therefore, in this study, we selected *ZNF*112/rs8113807 and *ZNF*180/rs2571051 as our research objects to analyze the relationship between them and patients with IVIG non-response, and achieved inspiring results. KD was an age-related disease. The incidence of KD was high in children under 5 years old, and it would be more serious in older children over 10 years old.[31,32] According to the results of this study, the risk effect of *ZNF112*/rs8113807 TC/CC vs TT and the *ZNF180*/rs2571051 TT/CT vs CC were significantly associated with children younger than 5 years (≤60 months). It partly justified the prevalence of KD among children under 5 years old and explained why the condition could be severe in older children.

Disease-related SNPs in most complex diseases (such as KD) were located in non-coding sequences and were thought to affect regulatory genetic elements, resulting in changes in transcriptional activity, splicing, and epigenetic markers. Some studies have shown that eQTL was closely related to biological factors such as cell type and activation state.[33,34] Genetic variations could regulate the expression of genes that affect phenotypes and susceptibility to complex diseases. In this study, the effects of rs2571051 on gene expression and splicing events were evaluated by GTEx database. The mRNA expression of rs2571051 in *ZNF180* (the TT genotype presented high expression and the TT genotype was a risk factor in our study) and splicing events were found. Biologically, the changes in *ZNF180* expression and splicing caused by rs2571051 may affect the regulation of immune inflammation, and then affect the treatment of IVIG in KD patients (IVIG resistance), but the specific mechanism is still unknown.

In the early years, our team has been focused on the progress of KD disease and the therapeutic effect, and achieved some exciting results. We found that single nucleotide polymorphisms (SNPs) of several genes (*P2RY12*, *MRP4* and *EIF2AK4*[35–37]) were closely associated with IVIG treatment efficacy in KD; interestingly, these genes played important roles in immune response and cardiovascular-related functions. This study was the first one to focus on the effect of ZNF family (*ZNF180* and *ZNF112*) on IVIG treatment resistance of KD. Our findings enriched the genetic background of IVIG resistance to risk and provide new insights into the treatment of KD, which might be a predictor of IVIG resistance in KD patients.

This study is to evaluate the relationship between *ZNF112* and *ZNF180* polymorphisms and IVIG resistance susceptibility in KD patients, but it still had the following limitations: Firstly, the sample size of children older than 60 months is not large enough, and it should be further expanded. Future studies with larger sample size and functional experiments should be conducted to confirm the results obtained in this study. Secondly, only age and gender were considered in stratified analysis, more factors such as medical history and medication environment should be considered. Thirdly, only two SNPs (rs8113807 and rs2571051) were evaluated in this study, and more SNPs should be included in future studies.

Summary up, we found that *ZNF112*/rs8113807 TC/CC genotype and *ZNF180*/rs2571051 TT/CT genotype were the risk factors for IVIG resistance of KD in southern Chinese. The two genotypes (*ZNF112*/rs8113807 TC/CC + *ZNF180*/rs2571051 TT/CT) indicated a higher risk for children with both genotypes (versus 0–1 genotype) in children younger than 60 months.

Ethics Approval and Consent to Participate

The research followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (2014073009, 2018052702, 2021093A01). All parents of patients knew the purpose of this study and signed informed consent forms.

Consent for Publication

All authors consent to publication.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest.

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