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CLINICAL RESEARCH ARTICLE

Association of *ITPKC* gene polymorphisms rs28493229 and rs2290692 in North Indian children with Kawasaki disease

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BACKGROUND: Single-nucleotide polymorphisms (SNPs) of several genes are linked to the etiopathogenesis of Kawasaki disease (KD). Association of SNPs of inositol 1,4,5-triphosphate-3-kinase C (*ITPKC*) gene with susceptibility to KD and coronary artery lesions (CALs) has been observed in children of certain ethnicities, but not from others. The present study was planned to explore this genetic association in the North Indian cohort.

METHODS: Fifty children with KD and 50 age- and sex-matched controls were studied for two SNPs (rs28493229 and rs2290692) of the *ITPKC* gene using polymerase chain reaction and restriction fragment length polymorphism. Findings were confirmed by Sanger sequencing. A meta-analysis was also carried out for GG and CC genotypes of the SNPs.

RESULTS: There was significant association between KD susceptibility and CG + GG genotype of rs2290692 ($p = 0.015$, odds ratio = 4.1, 95% confidence interval = 1.38–13.83). None of the single alleles or genotypes of the SNPs of *ITPKC* were, however, significantly associated with KD susceptibility. A meta-analysis also did not show any significant association of these SNPs to KD susceptibility.

CONCLUSIONS: Our findings suggest that *ITPKC* gene SNPs (rs28493229 and rs2290692) did not have a significant association with susceptibility to KD in children from North India. Larger multicentric studies incorporating different ethnicities are required to understand the genetic basis of KD.

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IMPACT:

- While SNP rs28493229 of the *ITPKC* gene is not found to be associated with susceptibility to KD, the combined genotype of SNP rs2290692 is shown to be associated.
- Impact of *ITPKC* gene SNP on KD is different across different races and ethnicities. We could find an association of the combined genotype of rs2290692 with it in the Indian population.
- This study highlights that phenotype and genotypic association of KD varies with ethnicities. Larger multicentric studies are required to reach a conclusion regarding the genetic association of KD.

INTRODUCTION

Kawasaki disease (KD) is one of the common childhood vasculitides and is being increasingly recognized as an important cause of acquired heart disease in young children.[1] The highest incidence of KD has been reported from Japan, Korea, and Taiwan.[2–4] Reports from several centers in India over the past two decades suggest that the incidence of KD may be increasing.[2,5,6] Two hospital-based studies from Chandigarh, India showed that KD is the most common cause of vasculitis in young children.[7–9] One of the main concerns in KD is its propensity to affect the heart and cardiovascular system and this can result in the development of coronary artery lesions (CALs).[10–12] Treatment with IVIG not only reduces the occurrence of CAL but also decreases myocardial inflammation.[13,14]

Although therapy of KD has evolved over the past three decades,[1,15,16] the etiology of this disease remains a mystery. Many etiological associations have been proposed for KD, but the cause and effect relationship has not yet been established.[17] Genetic association of KD has been suspected because of the high incidence of the disease in children in families of Japanese ancestry and siblings or offsprings of patients with KD.[18,19] Inositol 1,4,5 triphosphate 3-kinase C (*ITPKC*) is a negative regulator of Ca^{2+}/nuclear factor of activated T cell (NFAT) pathway in T cells.[20] Onouchi et al. reported the association of polymorphism of rs28493229 of *ITPKC* with susceptibility to KD and CAL in Japanese and American KD cohorts.[21] This finding was, however, not replicated in studies from other countries like China and Taiwan.[22,23] Similarly, while Peng et al. reported an association of polymorphism of rs2290692 of *ITPKC* with susceptibility to KD in China,[22] the finding was not replicated in studies from Taiwan and Korea.[24,25] There is no study on SNPs of *ITPKC* in association with KD from India to date. As the phenotype of KD in India is different,

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Table 1. (A) Primers and restriction enzymes for concerned SNPs. (B) Review of published literature on SNP rs28493229 in association with susceptibility to KD and CAL. (C) Review of published literature on SNP rs2290692 in association with susceptibility to KD and CAL.

(A)

Location	SNP	Primers (5′–3′)	Restriction enzyme	PCR product	Restricted fragments
Intron 1	rs28493229	F1: GCTTCTCCTCTGCCTCTTCTTT R1: GGAGGAGGTCAGTGGATGGA	*Ban*I	237 bp	G allele: 177 + 60bp C allele: 141 + 60 + 36 bp Undigested: 237 bp
3′-UTR	rs2290692	F2: GACCTGGCTATGGGTGCTG R2: TTACACCTCCTGCCTGACCC	*Ava*I	295 bp	G allele: 295 bp C allele: 150 + 145 bp Undigested: 295 bp

(B)

S.N.	Author (year) and ethnicity	No. of subjects (cases/controls)	Association of SNP rs28493229 with				
			Allele (%) in KD	Risk of KD	Risk of CAL	Other risks	Combined SNPs
1	Onouchi et al. (2008), Japanese[21]	637/1034	C = 22%; G = 78%	Present with C allele	Present with C allele	Family history	–
2	Chi et al. (2010), Taiwanese[23]	385/1158	C = 8%; G = 92%	No association	No association	–	–
3	Lin et al. (2011), Taiwanese[40]	280/492	C = 8%; G = 92%	Present with C allele	No association	BCG site erythema	–
4	Kuo et al. (2011), Taiwanese[41]	341/1,190	C = 8.1%; G = 91.9%	No association	No association	-	Meta-analysis: all Taiwanese study: OR 1.36; CI 1.12–1.66
5	Khor et al. (2011), European and Asian[36]	2173/9383	GWAS: C 20%, G 80%; Taiwan: C 8%, G 92%; Korea: C13%, G 87%	Present with C allele	Present with C allele	Family history	Meta-analysis: $p = 6.98 \times 10^{-12}$
6	Peng et al. (2012), Han Chinese[22]	223/318	C = 6%; G = 94%	No association	No association	–	–
7	Lou et al. (2012), Meta-analysis[37]	10 CCS and 2 TDT studies	–	–	–	Meta-analysis: this SNP increases the risk of KD but not CAL (heterogeneity in integrated analysis)	
8	Onouchi et al. (2013), Japanese[42]	204/947	C = 23.8%; G = 76.2%	Present in C allele	Present in the combined analysis only	No significant association with IVIG resistance	–
9	Yan et al. (2013), Chinese[34]	358/815	C = 6.4%; G = 93.6%	No association	No association	Significant role of rs1801274 (FCGR2A), rs2857151(HLA), and rs2254546 (BLK) in KD	
10	Natividad et al. (2013), Filipino[32]	17/26	C = 2.9%; G = 97.1%	No association	No association	Higher % of risk allele “C” among controls than in case postulated as protective	
11	Kuo et al. (2013), Taiwanese[43]	340/controls	CC = 0.68% CG + GG = 99.32%	No association	No association	No association	Overrepresentation of CC in IVIG non-responders
12	Wang et al. (2014), Chinese[35]	428/493	C = 7%; G = 93%	No association	No association	Combined rs2720378 (GC/CC) (CASP3), rs2069762 (CA/CC) (IL-2), and rs1561876 AA (STIM1) have higher KD risk	
13	Kim (2018), Korean[25]	299/210	C = 12%; G = 88%	Present with CG or CC genotype	Present with CG or CC genotype	ITPKC rs2561531 CC and SLC11A1 rs 17221959 CT—may exert a protective effect	
14	Our study (2019), (North-Western Indian)	50/50	C = 11%; G = 89%	No association	No association	None	

Table 1 continued

(C)							
(C)							
1	Peng et al. (2012), Han Chinese[22]	223/318	C = 52.47%; G = 47.53%	Present with the ancestral C allele	Present with the ancestral C allele	Overrepresentation of CC in IVIG non-responders	–
2	Kuo et al. (2014), Taiwan[24]	340/controls	C = 54.4%; G = 45.6%	No association	No association	rs7251246 was significantly associated with CAL	C/G/G/T/G haplotype of *ITPKC* (rs11673492, rs7251246, rs890934, rs2607420, rs2290692) associated more with CAL
3	Kim (2018), Korean[25]	299/210	C = 46.3%; G = 53.7%	No association	No association	rs2290692 SNPs were overrepresented in patients with high inflammatory markers.	
4	Our study (2019), North-Western Indian	50/50	C = 52%; G = 48%	Present with the G allele	No association	None	None

BCG Bacille Calmette-Guerin, *CCS* case–control study, *CAL* coronary artery lesions, *GWAS* genome-wide association studies, *IVIG* Intravenous immunoglobulin, *ITPKC* inositol 1,4,5-triphosphate 3-kinase C, *KD* Kawasaki disease, *NFAT* nuclear factor of activated T cells, *SNP* single-nucleotide polymorphism, *TDT* transmission/disequilibrium test, *STIM1* stromal interaction molecule, *PCR* polymerase chain reaction, *UTR* untranslated region.

it is important to study the genotype of KD in Indian children.[26–28] In this study, we have studied the SNPs rs28493229 at intron 1 and rs2290692 at 3′-UTR (untranslated region) of the *ITPKC* gene in North Indian KD patients (with or without CAL) and compared the results with healthy controls.

METHODS

Study population

This study was carried out in Pediatric Allergy Immunology Unit, Advanced Pediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, India. Our institute serves as a not-for-profit tertiary care referral center for North India. Patients and controls were of North Indian ethnicity. Fifty cases (25 KD patients without CAL and 25 KD patients with CALs) and 50 age- and sex-matched controls were selected for sampling over a period of 14 months (January 2018–April 2019). The mean (SD) age of children with KD was 3.9 (2.53) years, whereas the mean (SD) age of controls was 3.92 (2.46) years. There were 30 (60%) boys and 20 (40%) girls (M:F = 1.5:1) in both the KD group and control group.

Diagnosis of KD was based on American Heart Association (AHA) guidelines.[1,29] A CAL was defined as any coronary artery abnormality and included ectasia, dilatation (with the coronary *z*-score value of ≥2 but <2.5), and aneurysms (with the coronary *z*-score value of ≥2.5).[1] Written informed consent was taken from all the subjects for the purposes of this study. The study protocol was approved by Institute Ethics Committee and Institute Thesis Committee. The manuscript has been approved by Departmental Review Board. Two-dimensional echocardiographic coronary assessment of patients was carried out in the febrile phase and ~4–6 weeks later on follow-up.

Genetic study

Peripheral venous blood sample (2–3 ml) was drawn from cases and control subjects in ethylenediaminetetraacetic acid (EDTA) vacutainer.

Genomic DNA was extracted from peripheral blood using QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany). DNA quality was determined using 1% agarose gel electrophoresis, followed by staining with ethidium bromide. The purity of DNA was determined by taking optical density (OD) of samples at 260 and 280 nm using TECAN infinite M200 Pro with Nanoquant plate (Tecan group (Life-sciences and Diagnostics) AG, Switzerland) and was stored at −80 °C till further analysis.

Genotyping for SNPs rs28493229 and rs2290692 was carried out using PCR and restriction fragment length polymorphism (RFLP). 3′-UTR and intron 1 of *ITPKC* gene were amplified using PCR at controlled conditions using specific oligonucleotide primers as previously used by Peng et al.[22] PCR primers (Integrated DNA Technologies, Lowa), restriction enzymes (*Ban*I and *Ava*I) (New England Biolabs Inc., Massachusetts), length of the PCR products, and the digested fragments are shown in Table 1A. Genotyping was performed by the PCR-RFLP method. Primer sequence, restriction enzymes, and band size details have been mentioned in Table 1A. PCR cycle conditions for amplification are available on request.

Representative gel pictures showing the genotypes of both the SNPs have been given in Fig. 1a, b. PCR products of samples with the presence of C allele and G allele were confirmed with bidirectional Sanger sequencing (Applied Biosystems, Foster, CA) (Fig. 1c, d). Results were reproducible with no discrepancy in genotyping.

Meta-analysis

In addition, all previous studies on SNPs rs28493229 and rs2290692 of the *ITPKC* gene in association with susceptibility and coronary complication of KD were gathered. A meta-analysis was performed for genotypes of both SNPs and their association with susceptibility to KD.

Statistical analysis

Allele and genotype frequencies of SNPs were compared between patients with KD and control subjects. Similar comparisons were also carried out between patients of KD with and without CALs. The association of categorical variables with KD patients was analyzed using χ^2 test/Fisher's exact tests, whereas comparisons of quantitative variables between two study groups were carried out using an independent-sample *t* test (parametric) or Mann–Whitney *U* test (nonparametric). A *p* value of ≤0.05 was taken as significant. Data analysis was done using R software version 5.3.0 with RStudio IDE (The R Foundation) in Windows 10 platform.

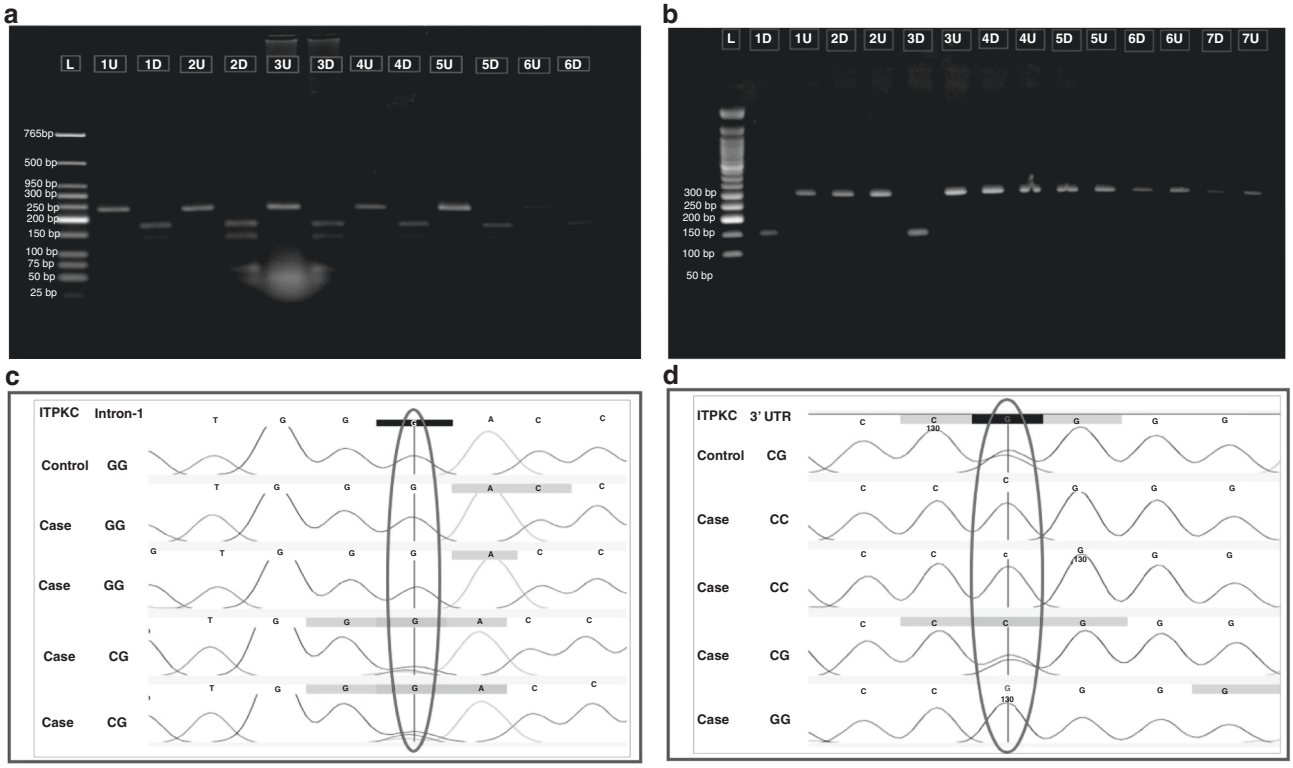

Fig. 1 Representative pictures of gel electrophoresis and sanger sequencing. a Gel electrophoresis for confirmation of CG genotypes with restriction fragments highlighted as bands of 36, 60, 141 and 177 bp in digested column and band of 237 bp in an undigested column of rs28493229 in intron 1 of ITPKC. **b** Gel electrophoresis picture showing bands of restriction fragments (digested and undigested columns) of rs2290692 in 3′-UTR of ITPKC. **c** Sanger sequencing of control and representative cases of different genotypes of rs28493229 at intron 1 of ITPKC. **d** Sanger sequencing results of control and representative cases of rs2290692 at 3′-UTR of ITPKC.

Deviation of SNPs from Hardy–Weinberg law was evaluated with Stata IC version 14 (StataCorp LLC, TX) using "genass" package. Meta-analyses were performed with R and Stata software.

RESULTS

Allele and genotype frequencies

Genotype distribution of SNPs rs28493229 (Pearson's χ^2 0.378, $p = 0.54$) and rs2290692 ($\chi^2 = 0.263$, $p = 0.76$) were in Hardy–Weinberg equilibrium. We analyzed the association of these two SNPs with KD and control groups. G and C allele frequency of rs28493229 observed among cases was 89% and 11%, respectively. Among controls, 92% G alleles and 8% C alleles were found (Table 2A). GG and CG genotype of this SNP was found to be 39 (78%) and 11 (22%) among cases. We could not get any case or control with the CC genotype of rs28493229 in our study. Similarly, the allele frequency of rs2290692 at 3′-UTR among cases was 63% and 37% for the G and C allele, respectively. Nineteen GG (38%), 25 CG (50%), and 6 CC (12%) genotypes of SNP rs2290692 were found in cases, whereas among controls, the frequencies were 20 (40%), 16 (32%), and 14 (28%), respectively. No statistical differences were found in allele, carrier, and genotype frequencies of both SNPs between cases and controls (Table 2A).

Association between ITPKC polymorphism and occurrence of KD

On multivariate logistic regression, adjusted odds of KD occurrence were increased by nearly one and half times (odds ratio (OR) 1.53; 95% confidence interval (CI): 0.54–4.65; $p = 0.43$) in subjects with CG genotype on rs28493229. Similarly, adjusted odds of KD occurrence were increased by 1.8 times (OR 1.8; 95% CI: 0.72–4.56; $p = 0.21$) in subjects with CG genotype on rs2290692. However, these associations failed to reach statistical significance (Table 2A). We observed an over-representation of CG genotype in rs28493229 as well as rs2290692 at 3′-UTR in patients with KD. Adjusted odds of occurrence of KD using SNP predictors are also demonstrated in a forest plot (Supplemental Fig. 1A).

Effects of combined genotypes for the risk of KD were also analyzed. On multivariate logistic regression, adjusted odds of occurrence of KD were increased by more than 4 times in subjects with any genotype with G allele (i.e., CG + GG) of rs2290692 (OR 4.14; 95% CI: 1.38–13.83; $p = 0.015$) after adjusting the confounding effects of sex and another SNP (Table 2C).

Association between ITPKC polymorphism and occurrence of coronary abnormality in KD

Allele, genotype, and carrier frequencies were analyzed between the KD patients with and without CALs. Effect size in genetic characteristics of both SNPs failed to reach statistical significance (Table 2B). Adjusted odds of occurrence of coronary abnormality in KD were lowered by 50% in subjects with CC genotype on rs2290692 at 3′-UTR (OR 0.50; 95% CI: 0.06–3.35; $p = 0.49$) after adjusting the confounding effects of sex and other SNPs (Supplemental Fig. 1B).

Association of ITPKC polymorphism and size of aneurysms

The *z*-scores for the size of aneurysms were divided into tertiles. The highest tertile scores represented higher *z*-scores. No significant association of SNPs was observed with the size of aneurysms. These effect sizes were adjusted for the age at which the illness occurred. Predictive margins of two SNPs with a probability of largest aneurysms (*z*-score) are illustrated in Supplemental Fig. 1C, D.

Table 2. (A) Allele, genotype, and carrier frequencies of single-nucleotide polymorphisms rs28493229 and rs2290692 in test subjects and controls. (B) Frequencies of allele and genotype of rs28493229 and rs2290692 SNPs and results of logistic regression analysis in KD patients with CAL and those without CAL. (C) Results of univariate as well as multivariate logistic regression analysis of combined genotypes with the dependent outcome as the occurrence of KD.

(A)

Allele, genotype, and carrier frequencies of SNP rs28493229 of ITPKC between patients with KD and control ($n = 100$)

Allele/genotype	KD patients ($n = 50$), n (%)	Controls ($n = 50$), n (%)	Univariate odds ratio (95% CI)	Multivariate odds ratio (95% CI)	p value
G allele	89 (89%)	92 (92%)	–	–	–
C allele	11 (11%)	8 (8%)	1.42 (0.49–4.26)	1.41 (0.46–2.56)	0.468
GG genotype	39 (78%)	42 (84%)	–	–	–
CG genotype	11 (22%)	8 (16%)	1.48 (0.54–4.19)	1.53 (0.53–4.57)	0.434
CC genotype	0 (0%)	0 (0%)	–	–	–
GG + CG	50 (100%)	50 (100%)	–	–	–
CC + CG	11 (22%)	8 (16%)	1.37 (0.45–4.28)	1.37 (0.42–4.59)	0.52
Frequencies of allele and genotype of rs2290692 SNPs in KD patients and controls ($n = 100$)					
Allele/genotype	KD patients ($n = 50$), n (%)	Controls ($n = 50$), n (%)	Odds ratio (95% CI)		p value
G allele	63 (63%)	56 (56%)	–		–
C allele	37 (37%)	44 (44%)	0.747 (0.41–1.36)	0.75 (0.39–1.49)	0.313
GG genotype	19 (38%)	20 (40%)	–	–	–
CG genotype	25 (50%)	16 (32%)	1.64 (0.68–4.04)	1.80 (0.72–4.56)	0.21
CC genotype	6 (12%)	14 (28%)	0.45 (0.14–1.37)	0.49 (0.14–1.53)	0.228
GG + CG	44 (88%)	36 (72%)	–	–	–
CC + CG	31 (62%)	30 (60%)	0.845 (0.41–1.74)	0.85 (0.39–1.82)	0.622

(B)

Frequencies of allele and genotype of rs28493229 SNPs and results of logistic regression analysis in KD patients with CAL and those without CAL

Allele/genotype	Frequency in KD without CAL ($n = 25$), n (%)	Frequency in KD with CAL ($n = 25$), n (%)	Univariate odds ratio (95% CI)	p value	Multivariate odds ratio (95% CI)	p value
G allele	45 (90%)	44 (88%)	–	–	–	–
C allele	5 (10%)	6 (12%)	0.83 (0.187–3.54)	0.77	0.8 (0.16–3.60)	0.80
GG genotype	20 (80%)	19 (76%)	–	–	–	–
CG genotype	5 (20%)	6 (24%)	1.26 (0.33–5.05)	0.733	1.46 (0.36–6.21)	0.597
CC genotype	0 (0%)	0 (0%)	–	–	–	–
GG + CG	25 (100%)	25 (100%)	–	–	–	–
CC + CG	5 (20%)	6 (24%)	0.833 (0.176–3.77)	0.78	0.81 (0.16–3.60)	0.82
Frequencies of allele and genotype of rs2290692 SNPs and results of logistic regression analysis in KD patients without CAL and those with CAL						
Allele/genotype	Frequency in KD without CAL ($n = 25$), n (%)	Frequency in KD with CAL ($n = 25$), n (%)	Univariate odds ratio (95% CI)	p value	Multivariate odds ratio (95% CI)	p value
G allele	31 (62%)	32 (64%)	–	–	–	–
C allele	19 (38%)	18 (36%)	1.08 (0.44–2.65)	0.835	1.01 (0.41–2.71)	0.885
GG genotype	10 (40%)	9 (36%)	–	–	–	–
CG genotype	11 (44%)	14 (56%)	1.41 (0.43–4.77)	0.571	1.38 (0.4–4.84)	0.610
CC genotype	4 (16%)	2 (8%)	0.56 (0.14–1.37)	0.173	0.50 (0.06–3.35)	0.492
GG + CG	21 (84%)	23 (92%)	–	–	–	–

Table 2 continued

(B)					
Frequencies of allele and genotype of rs28493229 SNPs and results of logistic regression analysis in KD patients with CAL and those without CAL					
Allele/genotype	**Frequency in KD without CAL ($n = 25$), *n* (%)**	**Frequency in KD with CAL ($n = 25$), *n* (%)**	**Univariate odds ratio (95% CI)**	***p* value**	**Multivariate odds ratio (95% CI)**	***p* value**
CC + CG	15 (60%)	16 (64%)	1.02 (0.370–2.84)	0.955	1.00 (0.39–2.80)	0.998
(C)						
Variable	**Controls ($n = 50$), *n* (%)**	**KD group ($n = 50$), *n* (%)**	**Univariate odds ratio (95% CI, *p* value)**		**Multivariate odds ratio (95% CI, *p* value)**	***p* value**
Sex—male	30 (60)	30 (60)	Base			
Sex—female	20 (40)	20 (40)	1.00 (0.45–2.23)		1.09 (0.47–2.54)	0.834
Non-C intron1	42 (84)	39 (78)	Base			
C intron1	8 (16)	11 (22)	1.48 (0.54–4.19)		1.52 (0.53–4.58)	0.439
Non-C 3′-UTR (GG)	21 (42)	19 (38)	Base			
C-3′-UTR (CC + CG)	29 (58)	31 (62)	1.18 (0.53–2.65)		1.91 (0.78–4.81)	0.16
Non-G 3′-UTR (CC)	15 (30)	6 (12)	Base			
G-3′-UTR (CG + GG)	35 (70)	44 (88)	3.14 (1.15–9.59)		4.16 (1.38–13.83)	0.015

CI confidence interval, *KD* Kawasaki disease, *CAL* coronary artery lesion, *non-C intron1* genotype without C allele at rs28493229, *C intron1* any genotype with C allele in rs28493229, *non-C 3′-UTR* genotype without C allele in rs2290692, *C-3′-UTR* genotype with C allele in rs2290692, *non-G 3′-UTR* any genotype without G allele in rs2290692, *G-3′-UTR* any genotype with G allele in rs2290692. *n* total numbers, *p* probability value

Meta-analyses. We further performed a meta-analysis for a possible association of both SNPs and susceptibility and complications of KD by combining the results of our study with all previous studies on rs28493229 and rs2290692 of the *ITPKC* gene. In the meta-analysis for the association of CC genotype of rs28493229, combined data confers a 1.5 times higher risk for this genotype to develop KD (OR = 1.46, 95% CI: 0.96–2.23) (Table 3A). Latter just crossed the line of null hypothesis showing only the trend for significance (Fig. 2a).

Similarly, a meta-analysis was performed individually for the association of CC and GG genotypes of rs2290692 with the risk of having KD. In the meta-analysis for the association of GG genotype of rs2290692, combined effect showed that odds of occurrence of GG in KD was 21% lower than in controls in this meta-analysis with an OR of 0.7881 and its 95% CI of 0.6085–1.0205 (Table 3B). The combined average only showed a trend for significance (Fig. 2b).

In the meta-analysis of CC genotype of our study and all previous studies of SNP rs2290692, combined OR did not confer any significant association with the susceptibility of KD (OR = 1.07, 95% CI: 0.663–1.731) (Table 3C and Fig. 2c).

DISCUSSION

KD is a common medium vessel vasculitis of young children. It may lead to complications like myocarditis, KD shock syndrome, and the development of CALs.[10,14] Etiology of KD still remains an enigma and its pathogenesis is poorly understood.[3,17,30] A high incidence of KD in some Asian populations, particularly Japanese and in native Japanese population residing in Hawaii, points towards some genetic link of KD.[3,19] A study by Onouchi et al. in 2008 revealed a significant association of polymorphisms of rs28493229 of *ITPKC* gene with susceptibility and severity of KD in Japanese and American patients.[21] Functional SNPs of *ITPKC* have been shown to result in altered messenger RNA (mRNA) splicing and gene transcription in patients with KD. Polymorphisms of *ITPKC* are reported as an important predisposing factor for KD.[21,31] We studied the association of two SNPs of the *ITPKC* gene in patients with KD and healthy controls of North Indian ethnicity.

Although certain polymorphisms of the *ITPKC* gene are shown to be associated with susceptibility to KD from some ethnicities, the results have not been uniformly replicated across ethnicities (Table 1B, C).[22–32] Such associations between SNP and KD need to be studied extensively in different ethnicities before coming to a conclusion. Moreover, the phenotype of KD in India is different.[26–28] Contrary to the pattern of KD in Eastern Asian and North American children, proportionately greater number of male patients, higher incidence of older children (almost half of them of age around 5 or more than that), early appearance of periungual peeling (prior to day 10 of fever), and early appearance of thrombocytosis were observed in Indian cohorts of KD. This suggests the possibility of different genotypic associations of these SNPs in patients with KD from India.

In the present study, we performed PCR-RFLP and representative bidirectional Sanger sequencing for two SNPs (rs28493229 at intron 1 and rs2290692 at 3′-UTR) of the *ITPKC* gene in North Indian children with KD. Diagnosis of KD was made on the basis of AHA guidelines.[1,29] According to the 1000 Genome Project, the prevalence of C and G allele of rs28493229 in the South Asian population is 13% and 87%, respectively.[33] In our study, C and G Allele frequency of rs28493229 in the KD group was 11% and 89%, respectively. Ancestral GG genotype of rs28493229 was found in 78% of patients with KD (Table 1B).

We failed to show an association of any allele or genotype of rs28493229 with susceptibility to disease. This result is in accordance with the findings of studies on Chinese, Taiwanese, and Filipino patients (Table 1B).[22,23,32,34,35] Although two previous meta-analyses had shown significant association of this SNP with KD,[36,37] our meta-analysis combining our study with all previous

Table 3. The meta-analysis of the association of different genotypes of SNPs of the *ITPKC* gene with KD.

Study (year) and country	CC in KD	KD (total)	CC in controls	Controls (total)	Risk ratio	Odds ratio	95% CI
(A) The meta-analysis of the association of CC genotype of SNP rs28493229 of *ITPKC* gene with KD							
Onouchi et al. (2008), Japan[21]	27	637	29	1034	1.512	1.535	0.904–2.606
Chi et al. (2010), Taiwan[23]	1	384	3	1158	1.286	1.287	0.189–8.751
Lin et al. (2011), Taiwan[40]	1	280	1	492	1.754	1.758	0.182–16.986
Kuo et al. (2011), Taiwan[41]	2	341	8	1131	0.993	0.993	0.241–4.092
Peng et al. (2012), China[22]	1	223	4	318	0.474	0.471	0.073–3.014
Yan et al. (2013), China[34]	3	358	5	815	1.446	1.450	0.377–5.573
Natividad (2013), Philippines[32]	0	17	0	26	1.500	1.514	1.514–0.028
Wang et al. (2014), China[35]	4	428	0	493	10.363	10.462	0.561–194.89
Kim et al. (2018), Korea[25]	4	299	0	210	6.330	6.411	0.343–119.71
Our study (2019), India	0	50	0	50	1.000	1.000	0.019–51.381
Combined	–	–	–	–	1.456	1.469	0.968–2.231
(B) The meta-analysis of the association of GG genotype of SNP rs2290692 of *ITPKC* gene with KD							
Peng et al. (2011), China[22]	54	223	116	318	0.663	0.556	0.379–0.815
Kuo et al. (2011), Taiwan[24]	73	381	122	569	0.893	0.868	0.627–1.201
Kim et al. (2018), Korea[25]	89	299	65	210	0.961	0.945	0.644–1.387
Our study (2019), India	19	50	20	50	0.950	0.919	0.411–2.054
Combined					0.845	0.788	0.608–1.020
(C) The meta-analysis of the association of CC genotype of SNP rs2290692 of*ITPKC* gene with KD							
Peng et al. (2011), China[22]	64	223	52	318	1.749	2.052	1.357–3.104
Kuo et al. (2011), Taiwan[24]	106	381	154	569	1.028	1.039	0.777–1.389
Kim et al. (2018), Korea[25]	67	299	48	210	0.978	0.972	0.639–1.480
Our study (2019), India	6	50	14	50	0.448	0.367	0.132–1.023
Combined					1.061	1.071	0.663–1.731

(A) The meta-analysis of the association of CC genotype of SNP rs28493229 of *ITPKC* gene with KD. (B) The meta-analysis of the association of the GG genotype of SNP rs2290692 of the *ITPKC* gene with KD. (C) The meta-analysis of the association of CC genotype of SNP rs2290692 of *ITPKC* gene with KD.

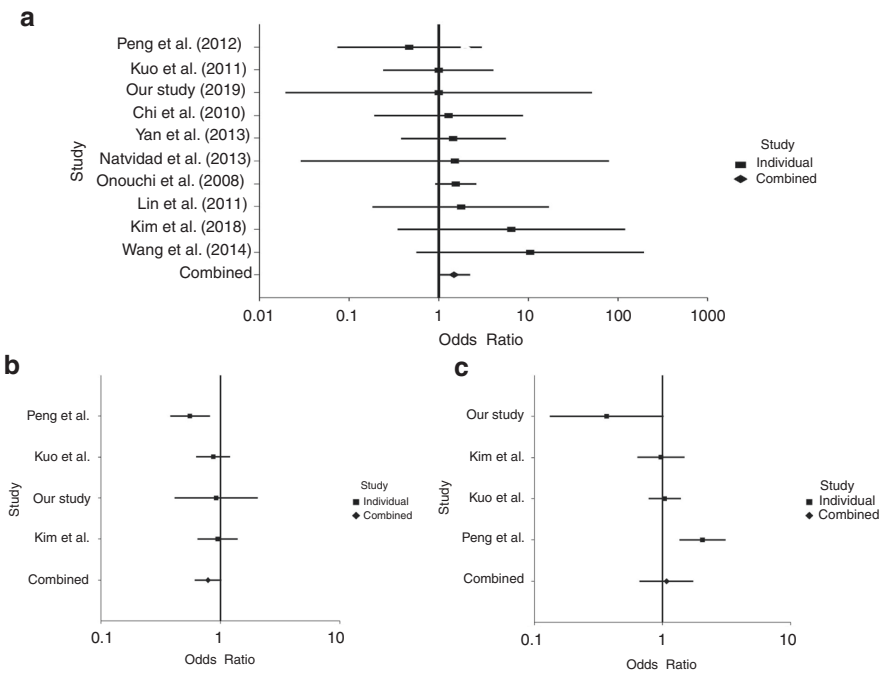

Fig. 2 Forest plot for the association of different genotypes of rs28493229 and rs2290692 of ITPKC gene. a Forest plot for the association between CC genotype of rs28493229 and risk of having KD (estimates of odds ratio and its 95% CI are plotted with a box and horizontal line). **b** Forest plot for the association between GG genotype of rs2290692 and risk of having KD (estimates of odds AQ8ratio and its 95% CI are plotted with a box and horizontal line). **c** Forest plot for the association between CC genotype of rs2290692 and risk of having KD (estimates of odds ratio and its 95% CI are plotted with a box and horizontal line).

studies on rs2849229 did not show any association with KD (Table 3A and Fig. 2a). This suggests that lowering of splicing efficiency of mRNA level due to the presence of the C allele of rs2849229 may not be a universal phenomenon.[21] This may also highlight the remarkable heterogeneity of functional SNP on KD across ethnicities.

Although the association of the C allele of rs2290692 of 3′-UTR and KD was reported from China,[22] our study did not show any association of any single allele or genotype with KD. Similar findings were reported in children with KD from Taiwanese and Korean ethnicities (Table 1C).[24,25] Meta-analyses combining our study with previous studies on rs2290692 also did not show any significant association with susceptibility to KD (Table 3B, C and Fig. 2b, c). This may simply reflect ethnic variation or the limited role of a single allele or genotype of the SNP on the pathogenesis of KD.

However, analyzing any genotype containing G allele (i.e., CG + GG) in rs2290692, we observed a statistically significant association of genotypes with G allele with KD occurrence in both univariate and multivariate analysis ($p = 0.015$; OR = 4.16, 95% CI: 1.38–13.83) (Table 2C). Such an association of rs2290692 is not described in previous studies. It is postulated that polymorphism in any allele may result in altered regulation efficiency of miRNAs. These miRNAs are involved in the regulation of gene expression. Our result showing an overrepresentation of the G allele in the KD group suggests that associated miRNAs may bind to the G allele altering the gene expression.[38] In addition, there may be evolutionary and epigenetic effects on the genetic structure of individuals in different ethnicities.

It appears that no specific SNP of the *ITPKC* gene may have a uniform association with susceptibility to KD and CAL formation across ethnicities. KD is undoubtedly a multifactorial disease and these SNPs may only confer some predisposition to develop KD.[39] Pathogenesis of KD may reflect the effect of several genes involved in calcium-NFAT signaling, which ultimately activates gene transcription for T cell activation and cytokine secretion.[35] Larger multicentric studies incorporating different ethnicities are required to understand the genetic basis of KD.

The relevance, strength, and limitations of the study

This is the first study of *ITPKC* SNP association with KD and CAL from India. Results from RFLP were confirmed by Sanger sequencing. Age and sex matching eliminate possible differences due to exposure prevalence and the effect of variable exposure in individuals. More importantly, the likelihood of false negatives due to the population stratification effect is also nullified by matching age and sex. However, this study has some limitations. This was a single-center hospital-based study and the numbers are understandably small. We were unable to conduct the functional level of studied gene polymorphism. Further, the effect of other genes (e.g., Ca^2+/NFAT or tumor growth factor-β pathway) in the studied cohort also cannot be excluded.

CONCLUSIONS

In conclusion, this is the first study in the Indian population that has explored the association of KD with two SNPs (rs28493229 and rs2290692) of the *ITPKC* gene. The frequency of the C allele of rs28493229 of *ITPKC* was found to be 11%, which is lesser than that in the Japanese population (22%), but comparable with KD cohorts from Taiwan and China. We documented a higher frequency of G allele (63%) of rs2290692 in patients with KD in our study. This study, and meta-analysis, did not provide any evidence to support the association of rs28493229 with KD susceptibility or CAL in children with KD of North Indian ethnicity. Combined genotypes containing the G allele of rs2290692 (CG + GG) were found to be significantly associated with the risk of KD but not with CAL. Further studies with a larger sample size are required to confirm this finding. In addition, studies on multiple SNPs of the *ITPKC* gene together with haplotype analysis are needed to explore the relevance of *ITPKC* gene polymorphisms in KD in the Indian population.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article (and its supplementary information file).

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AUTHOR CONTRIBUTIONS

D.B.: conception and design, acquisition of data, data analysis, data interpretation, drafting manuscript, editing, and critical revision. R.K.: acquisition of data and data analysis. A.K.: acquisition of data and data analysis. An.K.: concept and design of the study, data interpretation, editing of the draft, and critical revision. P.S.: data interpretation, editing draft, and critical revision. A.R.: design of the study, acquisition of data, and data analysis. S.S.: concept and design of the study, clinical data, editing, critical revision, and approval of the final version.

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COMPETING INTERESTS

The authors declare no competing interests.

CONSENT TO PARTICIPATE

Informed written consent is taken from parents of all subjects and controls. An assent was obtained from all children above 7 years of age. This study was approved by Institute Ethics Committee and Institute Thesis Committee and Departmental Review Board.

CONSENT FOR PUBLICATION

Yes.

ADDITIONAL INFORMATION

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