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Influence of IL-1β, STAT3 & 5 and TLR-5 gene polymorphisms on rheumatic heart disease susceptibility in north Indian population

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ABSTRACT

Background: Rheumatic heart disease (RHD) is the most serious complication of heart that comprises inflammatory reactions in heart valves. Many studies have demonstrated the contribution of host genetic factors in susceptibility to RHD and many cytokine gene variants have been linked with susceptibility to RHD. We sought to determine the role of genetic variants in IL-1β, STAT3, STAT5B and TLR5 genes in conferring risk of RHD in two cohorts of RHD patients.
Methods: The study included 400 echocardiography confirmed RHD patients and 300 controls from North Indian Population. We categorized RHD patients into two sub-groups based on involvement of heart valves, mitral valve lesion alone (MVL), and combined valve lesions including mitral valve (CVL). Genotyping for all the polymorphisms was done using TaqMan /PCR-RFLP methods.
Results: Our results showed that the genotypic frequencies of IL-1β, STAT3, STAT5B andTLR5 genes polymorphisms were significantly associated with RHD risk. To validate our results, we performed a replication study in additional 200 cases with similar clinical characteristics and results again confirmed consistent findings with RHD risk. In subgroup analysis, STAT3 polymorphism remained significant with MVL in RHD patients.
Conclusion: IL-1β, STAT3, STAT5B and TLR5 genes polymorphism may be useful markers for the identification of individuals with high risk of RHD in the susceptible population.

1. Introduction

Rheumatic Heart Disease (RHD) is the most serious consequence of rheumatic fever (RF) that follows group A streptococcal infection. The streptococcal M protein shares homology with human tissues, therefore molecular mimicry between antigens of the host and *Streptococcus pyogenes* has been proposed to trigger the disease [1]. In RHD, the heart develops calcification and leaflet thickening over time [2]. The estimated prevalence of RHD is 2.5–3.2cases per 1000 of the population and affects 15.6–19.6 million people worldwide [3]. The RHD is still common in developing countries like India. It is a major cause of valvular heart disease; the susceptible individuals develop carditis of varying severity after an acute infection episode of weeks or years and may damage heart valves. After recovery from the acute stage of carditis, disease may later advance to chronic RHD [4–6]. It has been reported that among patients dying of RHD, the mitral valve is most commonly afflicted either alone or in combination with the aortic and tricuspid valves [7]. The pathogenic mechanisms involved in the development of RF/RHD remain unclear; however, it is evident that an abnormal humoral and cellular immune response occurs. Many studies have demonstrated the contribution of host genetic factors in susceptibility to RHD, especially those involved in inflammatory and immune responses [8,9]. Elevated plasma levels of the pro-inflammatory cytokines, such as interleukin-1 (IL-1) had been reported in RF/RHD patients [10]. It is therefore postulated that polymorphisms in cytokine genes maybe predictive of an individual's predisposition to RHD. Previously, studies have reported association of many cytokine gene variants [11–13] with RHD.

The signal transducers and activators of transcription (STATs1–6) are intracellular effector molecules of cytokine-modulate signaling,

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which play important roles in the development of the human immune system and hematopoiesis, and are involved in the regulation of T-cell survival. Several studies reported association of JAK–STAT signaling dysfunction with many inflammatory and autoimmune diseases such as Atopic dermatitis (AD) [14], Crohn's disease (CD) [15], multiple sclerosis and Rheumatic Arthritis (RA) [16]. Role of STAT protein dysfunction in cardiovascular diseases has also been reported [17,18]. To date, the associations between STAT gene SNPs and susceptibility to autoimmune diseases have been known, and their role in RHD has also been reported [19].

Toll like Receptors (TLRs) are molecules of the innate immune system. The TLR5 gene product, in particular, recognizes bacterial flagellin [20,21] and is expressed in myelomonocytic cells, gut epithelial cells [22,23] and small intestine dendritic cells residing in the lamina propria [24]. Recognition of antigens have key role in innate mucosal immunity. Various cell surface receptors like TLRs recognize different microbial-associated molecular patterns (MAMPS), not expressed by the host but shared by many microbes [25]. R392X is a common (5% allele frequency) and stop polymorphism of TLR5 gene. It is a point mutation at nucleotide position 1174 which generates a stop codon rendering TLR5 inactive [26].

We have carried out the present study in two cohorts (primary cohort = 400 cases & 300 controls) and (replicative cohort = 200 cases & 300 controls) from North India. Results of primary cohort were validated in replication cohort and both cohorts' results were combined to derive combined risk. Genetic risk was also calculated for disease severity with involvement of only mitral valve lesion alone (MVL), and multiple valve lesions including mitral valve, combined valve lesions (CVL). We have chosen common variants among IL-1β, STAT3, STAT5B and TLR5 genes in the case control association study.

2. Materials and methods

2.1. Ethics statement

The institutional ethical committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS) approved the study protocol, and the authors followed the norms of World Medical Association Declaration of Helsinki. All the participants gave written informed consent for the study.

2.2. Study population

The present study was carried out in two stages, primary and replication stages. In the primary stage, we studied 400 RHD patients (218 males and 182 females, mean age 35.71 ± 12.68 years) and in the replication stage, further 200 cases (108 males and 92 females, mean age 33.82 ± 12.19 years) were enrolled. All the patients were recruited from the Department of Cardiology, SGPGIMS, Lucknow, and Uttar Pradesh, India. The diagnostic parameters used in the primary stage were also applied to the replication stage.

Both the primary and replication cohorts had significant RHD. The diagnosis was confirmed by echocardiography and severity of valve lesions was graded according to echocardiographic findings as mild, moderate or severe. The patients were further classified into MVL and CVL subgroups. In the primary cohort, the MVL group included 216 patients while the CVL group included 184 patients and in the replication cohort, the MVL group included 144 patients while the CVL group included 56 patients. Only patients who suffered from RF with valve involvements were included and patients with suspected or proven rheumatic fever RF without valve involvement were excluded from the study.

The control population consisted of 300 healthy controls (178 males and 122 females, mean age 37.36 ± 13.41 years). Controls had normal echocardiography and no family history of RHD. In addition, the inclusion criteria for controls were absence of any autoimmune disease and cardiac problems. All individuals were of North Indian origin. Both patient and control were frequency-matched to age, gender and ethnicity. All the participants gave written informed consent for the study. After obtaining informed consent, all the individuals' were personally interviewed for information.

2.3. Selected polymorphisms

IL-1β [rs2853550C/T], STAT3 C/G [rs4796793], STAT 5B C/T [rs6503691] and TLR5 (R392X) C/T were selected.

2.4. Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting out method [27]. The polymorphisms of different genes were genotyped using the PCR-RFLP/TaqMan Allelic Discrimination Assay methods. As a negative control, PCR mix without DNA sample was used to ensure contamination free PCR product. Samples that failed to genotype were scored as missing. Genotyping of IL-1β [rs2853550C/T], STAT 5B C/T [rs6503691] and TLR5 (R392X) C/T was performed by PCR/RFLP method [28,29] while genotyping of STAT3 C/G [rs4796793] was performed by TaqMan Allelic Discrimination Assay. Laboratory personnel were blinded to the case–control status of the subjects. The primer sequences of the polymorphism, restriction enzyme and technique used for the study are given in Supp. Table 1.

A PCR Reaction mixture of 25 μl volume was used for each PCR. We added 10 pmol of each primer, together with 50 ng genomic DNA into PCR mix (Lucigen Corporation, Middleton, WI, USA). The genomic DNA was amplified according to PCR conditions (Supp. Table 2), using the reaction mixture for IL-1β, STAT 5B and TLR5 gene polymorphism. Genomic DNA was amplified in Eppendorf TM Mastercycler ep Gradient S thermal cycler in 96-well format using specific primer sequences (Supp. Table 1). The amplified PCR product was checked by electrophoresis of 5 μl amplified PCR products on 2% agarose gel containing ethidium bromide. For detailed information regarding primer sequence, technique used, restriction enzyme (Fermantas INC, USA), amplification product and fragments size for IL-1β [rs2853550C/T], STAT3 C/G [rs4796793], STAT 5B C/T [rs6503691] and TLR5 (R392X) C/T please consider Supp. Table 1. Digestion was carried out overnight at 37 °C. The digested samples were run on 2% agarose gel containing ethidium bromide (0.5 μg/ml) and electrophoresis was done at 150 V (constant) for 2–4 h. Gel was visualized on UV transilluminator and photographed by Bio-rad GeldocTM Imager.

STAT3 C/G [rs4796793] was genotyped with the ABI TaqMan MGB diallelic discrimination system, using assay on demand (C_27977213_10, Applied Biosystems, and Foster City, CA, USA). The reactions were prepared using 2× TaqMan Universal Master Mix, 40× SNP Genotyping Assay Mix, DNase-free water, and10 ng genomic DNA in a final volume of 10 μl per reaction. The PCR amplification was performed using the ABI 7500 Fast Real-Time PCR system.

2.5. Statistical analysis

2.5.1. Single locus analysis

Descriptive statistics were presented as mean and standard deviation [SD] for continuous measures while absolute value and percentages were used for categorical measures. For observed genotype frequencies of all the polymorphisms in controls, the chi-square goodness of fit test was used for any deviation from Hardy Weinberg Equilibrium. Chi-square analysis or two-sided Fisher's exact test was used to compare the differences in demographic variables and genotype distributions of the polymorphisms between cases and controls. In addition, binary logistic regression was used to analyze the association between studied genes polymorphism and significant risk factors of variants, odds ratio (OR) and 95% confidence interval (CI) adjusted for age and gender. A p-value < .05 were considered statistically significant. The association between gene polymorphism and significant risk factors of RHD were analyzed using binary logistic regression. A two-tailed p-value of <.05 was considered a statistical significant result. All statistical analyses were performed using SPSS software version 16.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Patient characteristics

Demographic profile of Controls is shown in Supp. Table 3. Clinical characteristics of RHD patients – primary, replication and combined cohorts of study – are shown in Supp. Table 4. In combined cohort, there was no significant difference in the mean age of RHD patients and controls. The male/female ratio was comparable in both RHD cases as well as in controls. On evaluation of the valvular lesion in the combined cohort showed that 60% RHD patients were affected with MVL and 40% with CVL. All the values in primary stage matched to replication stage and there was no significant difference in the values of three stages of the study.

3.2. Single locus analysis of all studied polymorphisms

3.2.1. Influence of IL-1β C/Trs2853550, STAT3C/Grs4796793 & STAT 5BC/Trs6503691 and TLR5 (R392X) C/T on RHD

The genotypic distribution of IL-1β [rs2853550C/T], STAT3C/Grs4796793 & STAT 5BC/Trs6503691 and TLR5 (R392X) C/T between RHD patients (Primary, Replication and Combined stages) and controls are shown in Table 1. Distributions of genotypes of IL-1β [rs2853550C/T], STAT3C/Grs4796793 & STAT 5BC/Trs6503691 and TLR5 (R392X) C/T in controls were in accordance with Hardy-Weinberg equilibrium ($p = .98$).

Our results suggested significant association of IL-1β C/Trs2853550 genotype frequency with RHD patients (both stages) and controls

Table 1
Different Gene Polymorphism's genotype and allelic frequencies in controls and RHD patients (Primary cohort; patients = 400, replication cohort; patients = 200 & combined group = 600).

Genotypes	HC (300)	RHD[a] (400)	RHD[b] (200)	RHD[c] (600)	*p*-Value	*p*-Value	*p*-Value
	N (%)	N (%)	N (%)	N (%)	(95% CI)[a] OR	(95% CI)[b] OR	(95% CI)[c]OR
IL-1β C/T$_{rs2853550}$							
TT	126 (42.00%)	138 (34.50%)	68 (34.00%)	206 (34.3)	Ref.	Ref.	Ref.
CT	147 (49.00%)	193 (48.25%)	97 (48.50%)	290 (48.3)	0.28 (0.86–1.65)1.19	**0.02** (0.27–0.89)0.49	0.21 (0.89–1.62)1.20
CC	27 (9.00%)	69 (17.25%)	35 (17.50%)	104 (17.3)	**<0.01 (1.40–3.86)2.33**	0.20 (0.72–4.41)1.79	**<0.01 (1.46–3.79)2.35**
STAT3C/G$_{rs4796793}$							
CC	125 (41.7%)	127 (31.75%)	60 (30.00%)	187 (31.2%)	Ref.	Ref.	Ref.
CG	135 (45.0%)	200 (50.00%)	103 (51.50%)	303 (50.5%)	**0.03 (1.01–2.01)1.44**	**0.01 (1.16–3.78)2.10**	**<0.01 (1.10–2.03)1.50**
GG	40 (13.3%)	73 (18.25%)	37 (18.50%)	110 (18.3%)	**0.01 (1.12–2.81)1.78**	**<0.01 (1.39–7.18)3.16**	**<0.01 (1.20–2.81)1.83**
STAT 5BC/T$_{rs6503691}$							
CC	150 (50.00%)	133 (33.25%)	66 (33.00%)	199 (33.2%)	Ref.	Ref.	Ref.
CT	121 (40.30%)	207 (51.75%)	105 (52.50%)	312 (52.0%)	**<0.01 (1.39–2.66)1.92**	**0.01 (1.12–3.59) 2.01**	**<0.01 (1.44–2.61)1.94**
TT	29 (9.70%)	60 (15.00%)	29 (14.50%)	89 (14.8%)	**<0.01 (1.40–3.83)2.31**	0.12 (0.83–4.46)1.93	**<0.01 (1.44–3.70)2.31**
TLR5(R392X) C/T							
CC	288 (96.0%)	363 (90.8%)	182 (91.0%)	545 (90.8%)	Ref.	Ref.	Ref.
CT	11 (3.7%)	33 (8.2%)	16 (8.0%)	49 (8.2%)	**0.01 (1.14–4.67)2.31**	0.06 (0.94–4.89)2.15	**0.01 (1.16–4.47)2.27**
TT	1 (0.3%)	4 (1.0%)	2 (1.0%)	6 (1.0%)	0.28 (0.36–29.79)3.30	0.36 (0.27–4.20) 3.07	0.29 (0.37–26.11)3.12

RHD = rheumatic heart disease; HC = healthy control; N = number of controls and cases; RHD[a] = number of patients in primary cohort.
RHD[b] = number of patients in replication cohort; RHD[c] = number of patients in combined group.
p-Value/(95% CI) [a] OR = *p* value and Odds Ratio adjusted with 95% class interval of Healthy Control vs. Primary Cohort.
p-Value/(95% CI) [b] OR = *p* value and Odds Ratio adjusted with 95% class interval of Healthy Control vs. Replication Cohort.
p-Value/(95% CI) [c]OR = *p* value and Odds Ratio adjusted with 95% class interval of Healthy Control vs. Combined Group.

[Table 1 {Primary cohort; CC; *p* value < .01; OR (95% CI) = 2.33 (1.40–3.86) and Replication cohort; CT; *p* value = .02}] The frequency of rs2853550 in Combined RHD patients was also significantly higher [*p* value < .01; OR (95% CI) = 2.35 (1.46–3.79); Table 1] than controls.

STAT3C/Grs4796793 & STAT 5BC/Trs6503691 genotype frequencies showed significant association between RHD and controls in both cohorts [Table 1; STAT3C/Grs4796793; Primary Cohort {CG; *p* value = .03; OR (95% CI) = 1.44 (1.01–2.01) and GG; *p* value = .01;

Table 2
Genotype and allelic frequencies of IL-1β C/Trs2853550in MVD and CVD subgroups [Primary Cohort (*N* = 400), Replicative cohort (*N* = 200) and Combined Group (*N* = 600)].

Genotype	Control[a] *N* = 300 (%)	RHD patients		*p*-Value	*p*-Value	*p*-Value
		MVD[b]	CVD[c]	(95% CI) [(a&b)] OR	(95% CI)[(a&c)] OR	(95% CI)[(b&c)] OR
Primary Cohort (Total = 400;MVD = 216, CVD = 184)						
TT	126 (42.0%)	69 (31.9%)	69 (37.5%)	Ref.	Ref.	Ref.
CT	147 (49.0%0)	107 (49.5%)	86 (46.7%)	0.16 (0.89–1.94) 1.32	0.73 (0.72–1.59) 1.07	0.44 (0.54–1.31) 0.84
CC	27 (9.0%)	40 (18.5%)	29 (15.8%)	**<0.01 (1.56–4.90) 2.76**	**0.03 (1.07–3.56) 1.95**	0.32 (0.41–1.34) 0.74
Replicative cohort (Total = 200; MVD = 144, CVD = 56)						
TT	126 (42.0%)	48 (33.3)	20 (35.7)	Ref.	Ref.	Ref.
CT	147 (49.0%)	66 (45.8)	31 (55.4)	0.33 (0.48–1.27) 0.79	0.29 (0.74–2.69) 1.41	0.72 (0.57–2.21) 1.12
CC	27 (9.0%)	30 (20.8)	5 (8.9)	0.82 (0.58–1.96) 1.06	0.33 (0.20–1.71) 0.58	0.09 (0.13–1.17) 0.40
Combined Group (N = 600; MVD = 360, CVD = 240)						
TT	126 (42.0%)	117 (32.5%)	89 (37.1%)	Ref.	Ref.	Ref.
CT	147 (49.0%)	173 (48.1%)	117 (48.8%)	0.88 (0.59–1.55) 0.96	0.84 (0.56–2.03) 1.06	0.51 (0.61–1.27) 0.88
CC	27 (9.0%)	70 (19.4%)	34 (14.2%)	0.86 (0.57–1.92)1.05	0.47 (0.25–1.90) 0.69	0.07 (0.38–1.04) 0.63

Significant values are shown in BOLD.
MVL — Mitral Valve Lesion, CVL — Combined Valve Lesion.
a — Frequencies of control, b — Frequencies of MVL subgroup and c — Frequencies of CVL subgroup.
a&b — Analysis between controls and MVL subgroup.
a&c — Analysis between controls and CVL subgroup.
b&c — Analysis between MVL and CVL subgroup.

OR (95% CI) = 1.78 (1.12–2.81)}; Replication cohort {CG; *p* value = .01; OR (95% CI) = 2.10 (1.16–3.78) and GG; *p* value < .01; OR (95% CI) = 3.16 (1.39–7.18)} & STAT 5BC/Trs6503691; Primary Cohort {CT; *p* value < .01; OR (95% CI) = 1.92 (1.39–2.66) and TT; *p* value < .01; OR (95% CI) = 2.31 (1.40–3.83)}; Replication cohort {CT; *p* value = .01; OR (95% CI) = 2.01 (1.12–3.59)}]. The frequency of STAT3C/Grs4796793 & STAT 5BC/Trs6503691polymorphism in Combined RHD patients was also significantly higher [Table 1; STAT3C/Grs4796793; {CG; *p* value < .01; OR (95% CI) = 1.50 (1.10–2.03) and GG; *p* value < .01; OR (95% CI) = 1.83 (1.20–2.81)} & STAT 5BC/Trs6503691; {CT; *p* value < .01; OR (95% CI) = 1.94 (1.44–2.61) and TT; *p* value < .01; OR (95% CI) = 2.31 (1.44–3.70)}].

On comparison of genotype frequency of TLR5 (R392X) C/T between RHD patients and controls we found significant association of heterozygous variant CT with RHD [Table 1; Primary cohort; *p* value = .01; OR (95% CI) = 2.31 (1.14–4.67); Combined cohort; *p* value = .01; OR (95% CI) = 2.27 (1.16–4.47)].

3.2.2. Association of IL-1β C/Trs2853550, STAT3C/Grs4796793 & STAT 5BC/Trs6503691 and TLR5 (R392X) C/T with MVD and CVD subgroups

To find out whether IL-1β C/Trs2853550 associates with disease severity, we compared genotype frequencies of controls and patient subgroups i.e. MVD and CVD in Primary, Replication and Combined cohort (Table 2). Our results suggested that in Primary cohort, CC showed significant association with MVL and CVL subgroups [*p* value < .01; OR (95% CI) = 2.76 (1.56–4.90) and *p* value = .03; OR (95% CI) = 1.95 (1.07–3.56) Table 2].

For STAT3C/Grs4796793 & STAT5BC/Trs6503691, genotype frequencies between controls and MVD& CVD subgroups were compared in Primary, Replication and Combined cohorts (Table 3). We observed in Primary cohort, STAT3C/Grs4796793 & STAT5BC/Trs6503691 showed significant association with MVL subgroup [Table 3; rs4796793{CG; *p* value < .01; OR (95% CI) = 1.75 (1.18–2.61) and GG; *p* value < .01; OR (95% CI) = 2.18 (1.28–3.72)} & STAT 5BC/Trs6503691{CT; *p* value < .01; OR (95% CI) = 1.92

Table 3
Genotype and allelic frequencies of JAK-STAT Pathway Gene Polymorphism in MVD and CVD subgroups [Primary Cohort (N = 400), Replicative cohort (N = 200) and Combined Group (*N* = 600)]

Genotype	Control[a] N = 300 (%)	RHD patient MVD[b] N (%)	RHD patient CVD[c] N (%)	*p*-Value (95% CI)$^{(a\&b)}$ OR	*p*-Value (95% CI)$^{(a\&c)}$ OR	*p*-Value (95% CI)$^{(b\&c)}$ OR
Primary Cohort (Total = 400; MVD = 216, CVD = 184)						
STAT3C/G$_{rs4796793}$						
CC	125 (41.7%)	60(27.8%)	67(36.4%)	Ref.	Ref.	Ref.
CG	135 (45.0%)	114(52.8%)	86(46.7%)	**<0.01 (1.18–2.61) 1.75**	0.39 (0.79–1.77) 1.18	0.08 (0.43–1.05) 0.67
GG	40 (13.3%)	42(19.4%)	31(16.8%)	**<0.01 (1.28–3.72) 2.18**	0.19 (0.83–2.51) 1.44	0.17 (0.37–1.19) 0.66
STAT 5BC/T$_{rs6503691}$						
CC	150(50.00%)	72(33.3%)	61(33.2%)	Ref.	Ref.	Ref.
CT	121(40.30%)	112(51.9%)	95(51.6%)	**<0.01 (1.31–2.82) 1.92**	**<0.01 (1.29–2.88)1.93**	0.99 (0.64–1.90) 1.00
TT	29 (9.70%)	32(14.8%)	28(15.2%)	**<0.01 (1.29–4.08) 2.29**	**<0.01 (1.30–4.32)2.37**	0.91 (0.56–1.90) 1.03
Replicative cohort (Total = 200; MVD = 144, CVD = 56)						
STAT3C/G$_{rs4796793}$						
CC	125 (41.7%)	42(29.2%)	18(32.1%)	Ref.	Ref.	Ref.
CG	135 (45.0%)	70(48.6%)	33(58.9%)	0.06 (0.98–2.42) 1.54	0.09 (0.91–3.16) 1.69	0.77 (0.38–2.05) 0.88
GG	40 (13.3%)	32(22.2%)	5(8.9%)	**<0.01 (1.33–4.25) 2.38**	0.79 (0.30–2.48) 0.86	**0.04 (0.06–0.97) 0.24**
STAT 5BC/T$_{rs6503691}$						
CC	150(50.00%)	48(33.3%)	18(32.1%)	Ref.	Ref.	Ref.
CT	121(40.30%)	75(52.1%)	30(53.6%)	**<0.01 (1.25–2.99) 1.93**	**0.02 (1.099–3.88) 2.06**	0.85 (0.53–2.12) 1.06
TT	29 (9.70%)	21(14.6%)	8(14.3%)	**<0.01 (1.18–4.33) 2.26**	0.07 (0.91–5.78) 2.29	0.97 (0.38–2.70) 1.01

Genotype	Control[a] N = 300 (%)	RHD patient MVD[b] N (%)	*p*-Value CVD[c] N (%)	*p*-Value (95% CI)$^{(a\&b)}$ OR	*p*-Value (95% CI)$^{(a\&c)}$ OR	Genotype
Combined Group (N = 600; MVD = 360, CVD = 240)						
STAT3C/G$_{rs4796793}$						
CC	125 (41.7%)	102(28.3%)	85(35.4%)	Ref.	Ref.	Ref.
CG	135 (45.0%)	184(51.1%)	119(49.6%)	0.42 (0.49–1.35) 0.81	0.24 (0.76–2.86) 1.47	0.08 (0.49–1.04) 0.71
GG	40 (13.3%)	74(20.6%)	36(15.0%)	0.91 (0.51–1.81) 0.96	0.42 (0.21–1.90) 0.64	**<0.01 (0.30–0.83) 0.50**
STAT 5BC/T$_{rs6503691}$						
CC	150(50.00%)	120(33.3%)	79(32.9%)	Ref.	Ref.	Ref.
CT	121(40.30%)	187(51.9%)	125(52.1%)	0.98 (0.62–1.60) 1.00	0.84 (0.54–2.08) 1.07	0.93 (0.70–1.46) 1.01
TT	29 (9.70%)	53(14.7%)	36(15.0%)	0.96 (0.50–1.90) 0.98	0.94 (0.62–1.71) 1.03	0.90 (0.62–1.71) 1.03

Significant values are shown in BOLD.
MVL — Mitral Valve Lesion, CVL — Combined Valve Lesion.
a — Frequencies of control, b — Frequencies of MVL subgroup and c — Frequencies of CVL subgroup.
a&b — Analysis between controls and MVL subgroup.
a&c — Analysis between controls and CVL subgroup.
b&c — Analysis between MVL and CVL subgroup.

Table 4
Genotype and allelic frequencies of *TLR5 (R392X) C/T*in MVD and CVD subgroups.
[Primary Cohort (N = 400), Replicative cohort (N = 200) and Combined Group (N = 600)].

Genotypes	Control[a] N = 300 (%)	RHD patients		*p*-Value (95% CI)[(a&b)] OR	*p*-Value (95% CI)[(a&c)] OR	*p*-Value (95% CI)[(b&c)] OR
		MVD[b] N (%)	CVD[c] N (%)			
Primary Cohort (Total = 400; MVD = 216, CVD = 184)						
CC	288 (96.0%)	197 (91.2%)	166 (90.2%)	Ref.	Ref.	Ref.
CT	11 (3.7%)	17 (7.9%)	16 (8.7%)	**0.04 (1.03–4.92) 2.25**	**0.02 (1.14–5.56) 2.52**	0.75 (0.54–2.30) 1.12
TT	1 (0.3%)	2 (0.9%)	2 (1.1%)	0.38 (0.26–32.46) 2.92	0.31 (0.31–38.55) 3.47	0.77 (0.18–9.72) 1.33
Replicative cohort (Total = 200; MVD = 144, CVD = 56)						
CC	288 (96.0%)	128 (88.9%)	54 (96.4%)	Ref.	Ref.	Ref.
CT	11 (3.7%)	14 (9.7%)	2 (3.6%)	**0.01 (1.26–6.48) 2.86**	0.96 (0.20–4.49) 0.97	0.16 (0.07–1.54) 0.33
TT	1 (0.3%)	2 (1.4%)	0 (0.0%)	0.22 (0.40–50.07) 4.50	1.00 (0.00–0.00) 0.00	0.99 (0.00–0.00) 0.00
Combined Group (N = 600; MVD = 360, CVD = 240)						
CC	288 (96.0%)	325 (90.3%)	220 (91.7%)	Ref.	Ref.	Ref.
CT	11 (3.7%)	31 (8.6%)	18 (7.5%)	0.53 (0.60–2.66) 1.26	0.21 (0.08–1.72) 0.38	0.61 (0.46–1.57) 0.85
TT	1 (0.3%)	4 (1.1%)	2 (0.8%)	0.66 (0.21–11.06) 1.53	0.99 (0.00–0.00) 0.00	0.72 (0.13–4.06) 0.73

Significant values are shown in BOLD.
MVL — Mitral Valve Lesion, CVL — Combined Valve Lesion.
a — Frequencies of control, b — Frequencies of MVL subgroup and c — Frequencies of CVL subgroup.
a&b — Analysis between controls and MVL subgroup.
a&c — Analysis between controls and CVL subgroup.
b&c — Analysis between MVL and CVL subgroup.

(1.31–2.82) and TT; *p* value < .01; OR (95% CI) = 2.29 (1.29–4.08)}]. STAT5BC/Trs6503691 also showed significant association between controls and CVL subgroup [Table 3; {CT; *p* value < .01; OR (95% CI) = 1.93 (1.29–2.88) and TT; *p* value < .01; OR (95% CI) = 2.37 (1.30–4.32)}]In Replication Cohort, rs4796793 showed significant association with MVL subgroup [Table 3; rs4796793 {GG; *p* value < .01; OR (95% CI) = 2.38 (1.33–4.25)}]; also with MVL & CVL subgroup [Table 3; rs4796793 {GG; *p* value = .04}] whereas for STAT 5BC/Trs6503691 significant association was found between controls and MVL subgroup; controls and CVL subgroup[Table 3 {CT; *p* value < .01; OR (95% CI) = 1.93 (1.25–2.99) and TT; *p* value < .01; OR (95% CI) = 2.26 (1.18–4.33)} & {CT; *p* value = .02; OR (95% CI) = 2.06 (1.099–3.88)}]. In Combined cohort STAT3C/Grs4796793 [Table 3; {GG; *p* value < .01}] showed significant association with MVL and CVL subgroup.

On comparison of genotype frequencies of TLR5 (R392X) C/T between controls and MVD & CVD subgroups in Primary, Replication and Combined cohort (Table 4) significant association was found between controls and MVL; controls and CVL subgroups [Table 4; Primary cohort; {controls and MVL; *p* value = .04; OR (95% CI) = 2.25 (1.03–4.92) & controls and CVL; *p* value = .02; OR (95% CI) = 2.52 (1.14–5.56)} and Replication cohort {controls and MVL; *p* value = .01; OR (95% CI) = 2.86 (1.26–6.48)}].

4. Discussion

Using single analytic strategy with a panel of genetic polymorphisms involved in inflammatory and autoimmunity pathways, we have re-examined the associations of IL-1β, STAT3, STAT5B and TLR5 polymorphisms with RHD susceptibility. In single-locus analysis, these polymorphisms showed significant association with RHD susceptibility in both cohorts (Primary and Replication).

RHD is a complex disease with multiple pathways. It is well established that RHD, is an inflammatory, autoimmune disease; occurring as a consequence of group A streptococcal infection complicated by RF. An inappropriate immune response is the central signature tune to the complex pathogenesis of RHD. Genetic studies on RHD have provided evidences that genes associated with inflammatory pathways (TGF beta 1, TNF-alpha, IL-1 Ra, IL-4, IL-6, IL-10, CTLA-4, TLRs, and MBL etc.) impact the risk of disease initiation, progression, and severity. It is now proven that cytokines have critical roles in regulating immunity and inflammation. The STATs, including STATs 1–6 are intracellular effector molecules of cytokine-modulated signaling, which play important roles in the development of the human immune system and hematopoiesis, and are involved in the regulation of T-cell survival. Therefore based on these evidences, in the present study we have selected genetic variants (SNPs) from genes of inflammatory pathway as well as JAK–STAT signaling pathway in conferring risk of RHD.

In the present study, IL-1β rs2853550 showed significant association with RHD susceptibility and severity in Primary, Replication and Combined cohorts in North Indian population. Previously, Gupta et al. [30], showed IL-1β rs2853550 to confer significantly higher susceptibility for RHD in North Indian population. However Chou et al. [31] found no association between the IL-1β gene polymorphisms and RHD in Chinese population. In a meta-analysis study by Bhatt et al. [32], significant association was found between RHD risk and IL-1β gene polymorphism, in subjects with Asian ethnicity.

Pociot et al. [33] in a population-based analysis of unrelated individuals with IL-1β polymorphism showed an association between this polymorphism and IL-1β secretion after lipopolysaccharide stimulation studied by analyzing the in vitro stimulated monocyte IL-1β response. Therefore, the possible role of IL-1β gene polymorphism may influence RHD susceptibility through regulating IL1β secretion.

Inflammatory cytokines are known to be involved in the pathogenesis of a number of autoimmune and multi systemic inflammatory diseases [34–39]. The cytokine may be responsible for the increased valvular fibrosis and calcification in the pathogenesis of RHD. Rheumatic involvement is present in 99% of stenotic mitral valves excised at the time of mitral valve replacement [40]. As the basic rheumatic process is inflammation and destruction of connective tissue, the effects of interleukin may be involved in the pathogenesis of RHD.

STATs proteins are part of the JAK-STAT pathway mediating the transduction of stress signals from the plasma membrane to the nucleus [15,41,42] & [43]. STAT1–6 have been identified and are expressed in the heart, where they are detected in endothelial cells, in smooth

muscle cells, in cardiac fibroblasts, and in cardiomyocytes [41,44] & [45]. The location of STAT3 rs4796793 is 5upstream region and STAT5B rs6503691 is intronic region at chromosome 17. Ito et al. [46] observed significant correlation between STAT3 expression and rs4796793SNP. They showed that minor allele of rs4796793 SNP had a tendency for lower STAT3 expression. It is possible that lower expression of STAT3carriers may result in reduced production of anti-inflammatory cytokines. Subsequently, low levels of anti-inflammatory cytokines slowly cause damage to heart valves. The associations between STATs gene polymorphisms and multiple diseases have been widely studied [15,17,47–51] & [52]. Denise Hilfiker-Kleiner [53] reported impaired that down regulation of gp130-mediated STAT3 activation in subacute infarction promotes cardiac inflammation, adverse remodeling, and heart failure. Peng et al. [18] also stated that reduced STAT3 activation may play an important pathophysiological role in the development of DCM in Chinese population. The present results show that rs6503691 and rs4796793 are associated with RHD in Primary and Replication cohorts. Since STAT3 polymorphism is involved in severity of RHD as both MVL and CVL patients are equally affected. Thus, STAT genes may play important role in initiation and progression of RHD.

TLRs play essential role in pathogen recognition and activation of innate immune responses. There are currently 11 known mammalian TLRs. They are transmembrane receptors that are found either on the cell membrane (TLR1, 2, 4, 5 and 9) or on intracellular organelles (TLR3, 7 and 8) [54,55]. TLR5 is mapped to chromosome 1q41, containing 6 exons [56]. R392X is stop polymorphism of TLR5 gene. In human African and Eurasian populations, the polymorphism is found at low frequency of 12% [57].

Our results suggested significant association of TLR5 (R392X) with RHD susceptibility. In a study by Zhu et al. [58] polymorphism in TLR5 gene was associated with RHD among the Chinese Han population. In a study by Al-Daghri et al. [59] R392X was found significantly associated with type 2 diabetes (T2D) in a Saudi Arabia population. In a North Indian population based case control study by Meena et al. [60], R392X polymorphism was found significantly associated with the Ulcerative Colitis (UC). A study from Jewish population [61] shows that R392X have 6% and 0.9% heterozygosity in UC and CD (Crohn's disease) patients as compared to unaffected relatives (5.4%) and unrelated controls (6.5%). The risk for urinary tract infection in adult women was associated with R392X as concluded in previous studies [62]. It can affect lung function in cystic fibrosis patients as well [63].

In conclusion, overall our results suggest that genetic polymorphism in *IL-1β* −511C/T plays major role among inflammatory pathway genes in predisposition of RHD in north Indian population. We also demonstrate that STAT gene variants rs4796793 and rs6503691 are associated with an increased risk of RHD. This is the first case-control study to investigate the association of a common TLR5 (R392X) with RHD in North Indian population. Significant association between TLR5 (R392X) and RHD is observed. It implies that common genetic variants of IL-1β, STAT3, STAT5B and TLR5 genes could increase susceptibility to RHD. These findings add new evidence to existing data on the linkage between IL-1β, STAT3, STAT5B and TLR5 genes function and genetic predisposition to RHD. Therefore, IL-1β, STAT3, STAT5B and TLR5 genes polymorphism can be explored as biomarkers for identification of individuals likely to develop RHD in the susceptible population.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.03.035.

References

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[1] L. Guilherme, K. Fae, S.E. Oshiro, J. Kalil, Molecular pathogenesis of rheumatic fever and rheumatic heart disease, Expert Rev. Mol. Med. 7 (2005) 1–15.
[2] N.M. Rajamannan, F. Antonini-Canterin, L. Moura, J.L. Zamorano, R.A. Rosenhek, P.J. Best, et al., Medical therapy for rheumatic heart disease: is it time to be proactive rather than reactive? Indian Heart J. 61 (2009) 14–23.
[3] K. Roberts, S. Colquhoun, A. Steer, B. Remenyi, J. Carapetis, Screening for rheumatic heart disease: current approaches and controversies, Nat. Rev. Cardiol. 10 (2013) 49–58.
[4] J.R. Carapetis, A. Brown, N.J. Wilson, K.N. Edwards, Rheumatic fever guidelines writing G. An Australian guideline for rheumatic fever and rheumatic heart disease: an abridged outline, Med. J. Aust. 186 (2007) 581–586.
[5] E. Atalar, S.L. Tokgozoglu, M. Alikasifoglu, K. Ovunc, S. Aksoyek, S. Kes, et al., Angiotensin-converting enzyme genotype predicts valve damage in acute rheumatic fever, J. Heart Valve Dis. 12 (2003) 7–10.
[6] M.M. Morsy, N.A. Abdelaziz, A.M. Boghdady, H. Ahmed, E.M. Abu Elfadl, M.A. Ismail, Angiotensin converting enzyme DD genotype is associated with development of rheumatic heart disease in Egyptian children, Rheumatol. Int. 31 (2011) 17–21.
[7] P. Chopra, M.L. Bhatia, Chronic rheumatic heart disease in India: a reappraisal of pathologic changes, J. Heart Valve Dis. 1 (1992) 92–101.
[8] L. Guilherme, R. Ramasawmy, J. Kalil, Rheumatic fever and rheumatic heart disease: genetics and pathogenesis, Scand. J. Immunol. 66 (2007) 199–207.
[9] M.E. Engel, R. Stander, J. Vogel, A.A. Adeyemo, B.M. Mayosi, Genetic susceptibility to acute rheumatic fever: a systematic review and meta-analysis of twin studies, PLoS One 6 (2011), e25326. .
[10] L. Guilherme, J. Kalil, Rheumatic fever and rheumatic heart disease: cellular mechanisms leading autoimmune reactivity and disease, J. Clin. Immunol. 30 (2010) 17–23.
[11] N. Duzgun, T. Duman, F.E. Haydardedeoglu, H. Tutkak, Cytotoxic T lymphocyte-associated antigen-4 polymorphism in patients with rheumatic heart disease, Tissue Antigens 74 (2009) 539–542.
[12] A. Settin, H. Abdel-Hady, R. El-Baz, I. Saber, Gene polymorphisms of TNF-alpha (-308), IL-10(-1082), IL-6(-174), and IL-1Ra(VNTR) related to susceptibility and severity of rheumatic heart disease, Pediatr. Cardiol. 28 (2007) 363–371.
[13] S. Rehman, N. Akhtar, N. Saba, S. Munir, W. Ahmed, A. Mohyuddin, et al., A study on the association of TNF-alpha(-308), IL-6(-174), IL-10(-1082) and IL-1Ra(VNTR) gene polymorphisms with rheumatic heart disease in Pakistani patients, Cytokine. 61 (2013) 527–531.
[14] L. Bao, H. Zhang, L.S. Chan, The involvement of the JAK-STAT signaling pathway in chronic inflammatory skin disease atopic dermatitis, Jak-Stat. 2 (2013), e24137.
[15] L.R. Ferguson, D.Y. Han, A.G. Fraser, C. Huebner, W.J. Lam, A.R. Morgan, et al., Genetic factors in chronic inflammation: single nucleotide polymorphisms in the STAT-JAK pathway, susceptibility to DNA damage and Crohn's disease in a New Zealand population, Mutat. Res. 690 (2010) 108–115.
[16] L.B. Ivashkiv, X. Hu, The JAK/STAT pathway in rheumatoid arthritis: pathogenic or protective? Arthritis Rheum. 48 (2003) 2092–2096.
[17] L. Zhang, W.H. Kao, Y. Berthier-Schaad, Y. Liu, L. Plantinga, B.G. Jaar, et al., Haplotype of signal transducer and activator of transcription 3 gene predicts cardiovascular disease in dialysis patients, J. Am. Soc. Nephrol. 17 (2006) 2285–2292.
[18] Y. Peng, B. Zhou, Y. Wang, Y. Chen, H. Li, Y. Song, et al., Association between polymorphisms in the signal transducer and activator of transcription and dilated cardiomyopathy in the Chinese Han population, Mol. Cell. Biochem. 360 (2012) 197–203.
[19] U. Gupta, S.S. Mir, A. Srivastava, N. Garg, S.K. Agarwal, S. Pande, et al., Signal transducers and activators of transcription (STATs) gene polymorphisms related with susceptibility to rheumatic heart disease in north Indian population, Immunol. Lett. 161 (2014) 100–105.
[20] E. Lien, R.R. Ingalls, Toll-like receptors, Crit. Care Med. 30 (2002) S1–S11.
[21] E. Lien, R.R. Ingalls, Toll-like receptors, Crit. Care Med. 30 (2002) S1–11.
[22] A.T. Gewirtz, T.A. Navas, S. Lyons, P.J. Godowski, J.L. Madara, Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression, J. Immunol. 167 (2001) 1882–1885.
[23] S.H. Rhee, H. Kim, M.P. Moyer, C. Pothoulakis, Role of MyD88 in phosphatidylinositol 3-kinase activation by flagellin/toll-like receptor 5 engagement in colonic epithelial cells, J. Biol. Chem. 281 (2006) 18560–18568.
[24] S. Uematsu, K. Fujimoto, M.H. Jang, B.G. Yang, Y.J. Jung, M. Nishiyama, et al., Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing toll-like receptor 5, Nat. Immunol. 9 (2008) 769–776.
[25] B. Beutler, K. Hoebe, X. Du, R.J. Ulevitch, How we detect microbes and respond to them: the Toll-like receptors and their transducers, J. Leukoc. Biol. 74 (2003) 479–485.
[26] T.R. Hawn, A. Verbon, K.D. Lettinga, L.P. Zhao, S.S. Li, R.J. Laws, et al., A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease, J. Exp. Med. 198 (2003) 1563–1572.

[27] S.A. Miller, D.D. Dykes, H.F. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells, Nucleic Acids Res. 16 (1988) 1215.
[28] H. Kamal, G. Hussein, H. Hassoba, N. Mosaad, A. Gad, M. Ismail, Transforming growth factor-beta1 gene C-509T and T869C polymorphisms as possible risk factors in rheumatic heart disease in Egypt, Acta Cardiol. 65 (2010) 177–183.
[29] H.T. Chou, C.H. Chen, C.H. Tsai, F.J. Tsai, Association between transforming growth factor-beta1 gene C-509T and T869C polymorphisms and rheumatic heart disease, Am. Heart J. 148 (2004) 181–186.
[30] U. Gupta, S.S. Mir, N. Garg, S.K. Agarwal, S. Pande, B. Mittal, Association study of inflammatory genes with rheumatic heart disease in north Indian population: a multi-analytical approach, Immunol. Lett. 174 (2016) 53–62.
[31] H.T. Chou, C.H. Tsai, W.C. Chen, F.J. Tsai, Lack of association of genetic polymorphisms in the interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 genes with risk of rheumatic heart disease in Taiwan Chinese, Int. Heart J. 46 (2005) 397–406.
[32] M. Bhatt, S. Kumar, M.H. Siddiqui, N. Garg, B. Mittal, Influence of cytokine gene polymorphism on the risk of rheumatic heart disease — a meta-analysis, Immunol. Lett. 194 (2018) 69–78.
[33] F. Pociot, J. Molvig, L. Wogensen, H. Worsaae, J. Nerup, A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro, Eur. J. Clin. Investig. 22 (1992) 396–402.
[34] K. Morris, C. Mohan, P.L. Wahi, I.S. Anand, N.K. Ganguly, Enhancement of IL-1, IL-2 production and IL-2 receptor generation in patients with acute rheumatic fever and active rheumatic heart disease; a prospective study, Clin. Exp. Immunol. 91 (1993) 429–436.
[35] O. Yegin, M. Coskun, H. Ertug, Cytokines in acute rheumatic fever, Eur. J. Pediatr. 156 (1997) 25–29.
[36] L. Guilherme, P. Cury, L.M. Demarchi, V. Coelho, L. Abel, A.P. Lopez, et al., Rheumatic heart disease: proinflammatory cytokines play a role in the progression and maintenance of valvular lesions, Am. J. Pathol. 165 (2004) 1583–1591.
[37] F. Andreotti, I. Porto, F. Crea, A. Maseri, Inflammatory gene polymorphisms and ischaemic heart disease: review of population association studies, Heart. 87 (2002) 107–112.
[38] A. Cantagrel, F. Navaux, P. Loubet-Lescoulie, F. Nourhashemi, G. Enault, M. Abbal, et al., Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis, Arthritis Rheum. 42 (1999) 1093–1100.
[39] J. Grove, A.K. Daly, M.F. Bassendine, E. Gilvarry, C.P. Day, Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease, Gut. 46 (2000) 540–545.
[40] L.J. Olson, R. Subramanian, D.M. Ackermann, T.A. Orszulak, W.D. Edwards, Surgical pathology of the mitral valve: a study of 712 cases spanning 21 years, Mayo Clin. Proc. 62 (1987) 22–34.
[41] K. Boengler, D. Hilfiker-Kleiner, H. Drexler, G. Heusch, R. Schulz, The myocardial JAK/STAT pathway: from protection to failure, Pharmacol. Ther. 120 (2008) 172–185.
[42] P. Fischer, D. Hilfiker-Kleiner, Survival pathways in hypertrophy and heart failure: the gp130-STAT3 axis, Basic Res. Cardiol. 102 (2007) 279–297.
[43] P. Fischer, D. Hilfiker-Kleiner, Survival pathways in hypertrophy and heart failure: the gp130-STAT axis, Basic Res. Cardiol. 102 (2007) 393–411.
[44] J.E. Darnell Jr., Joys and surprises of a career studying eukaryotic gene expression, J. Biol. Chem. 288 (2013) 12957–12966.
[45] J.E. Darnell Jr., STATs and gene regulation, Science. 277 (1997) 1630–1635.
[46] N. Ito, M. Eto, E. Nakamura, A. Takahashi, T. Tsukamoto, H. Toma, et al., STAT3 polymorphism predicts interferon-alfa response in patients with metastatic renal cell carcinoma, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 25 (2007) 2785–2791.
[47] S. Kreil, K. Waghorn, T. Ernst, A. Chase, H. White, R. Hehlmann, et al., A polymorphism associated with STAT3 expression and response of chronic myeloid leukemia to interferon alpha, Haematologica. 95 (2010) 148–152.
[48] A. Vaclavicek, J.L. Bermejo, R.K. Schmutzler, C. Sutter, B. Wappenschmidt, A. Meindl, et al., Polymorphisms in the Janus kinase 2 (JAK)/signal transducer and activator of transcription (STAT) genes: putative association of the STAT gene region with familial breast cancer, Endocr. Relat. Cancer 14 (2007) 267–277.
[49] S. Sookoian, G. Castano, T.F. Gianotti, C. Gemma, M.S. Rosselli, C.J. Pirola, Genetic variants in STAT3 are associated with nonalcoholic fatty liver disease, Cytokine. 44 (2008) 201–206.
[50] R.R. Finan, F.E. Mustafa, I. Al-Zaman, S. Madan, A.A. Issa, W.Y. Almawi, STAT3 polymorphisms linked with idiopathic recurrent miscarriages, Am. J. Reprod. Immunol. 63 (2010) 22–27.