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Lack of association of TNFAIP3 polymorphism with rheumatic heart disease in Saudi population

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Abstract: *Rheumatic heart disease (RHD) is an autoimmune disease developed subsequently to Streptococcus infection. RHD is still causing acquired heart problems in children in many developing countries. TNFAIP3 encodes the ubiquitin-modifying enzyme (A20), an important negative regulator in different inflammatory pathways. Polymorphisms in TNFAIP3 have been reported to be associated with several inflammatory diseases. Tagging single nucleotide polymorphisms (tSNP), rs2230926, on chromosome 6q23, have been associated with RHD in Chinese population. In our study we evaluated the association of TNFAIP3 tSNP rs2230926 with RHD in Saudi population. TNFAIP3 tSNP was studied in 124 RHD patients and 205 controls by TaqMan allelic discrimination assay. Carditis was found in 100% in our patients, while arthritis was found in 53%. The genotype frequency of the polymorphism (TT, TC, and CC) in the control group was 58, 33, and 9%, and in the patient group was 48, 40, and 11%, respectively. There was no significant differences. Furthermore, subgroup analysis for the patients found no significant differences between the subgroups and controls. Our data suggest that TNFAIP3 tSNP rs2230926 examined in this study has no role in predicting the occurrence and severity of RHD in Saudi patients.*

Running Title: *TNFAIP3 polymorphism in Saudi Arabian rheumatic heart disease patients*
Keywords: *RF; RHD; TNFAIP3 polymorphism; Saudi Arabia*

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I. Introduction

Rheumatic fever [1] is a systemic illness that gives rise to a generalized inflammatory reaction affecting different body organs as the heart, joints, brain, skin and subcutaneous tissues. [2]It happens following throat infection with group A beta hemolytic streptococcal (GABHS) pharyngitis in those who were improperly treated or untreated at all. Autoimmune mechanism with cross reactivity with body's own tissues instead of bacterial antigen is supposed to be the most accepted explanation for subsequent tissue damage. [3]

Rheumatic cardiac affection is a cause of long term acquired cardiac disease30% to 50% ofchildrenand young adults with RF. [4] It is common in low and developed countries and among developing countries.[5, 6]In developing countries,RHDwas found to be responsible for more than 30%of hospital admissions by cardiaccauses.By the age of 15, more than 20 % of children with RHD will die if they did not receive treatment and the ratio will rise to be more than 70 % by age 25. [7]

In Saudi Arabia, the mean annual occurrence rate of acute RF was estimated in the Eastern District of the country, between 1 January 1980 and 31 December 1984 in arab children aged between 5 and 14 years old, and was 22 per 100,000 and 60 % of them were presented with carditis. [8]Whereas in another study from Western District of Saudi Arabia in 1987, the prevalence of rheumatic heart disease (RHD) was found to be as high as 240 per 100,000 among schoolchildren aged 6 to 15 years, it was more prevalent in female in rural areas and in low socioeconomic level. [9] A third study, conducted over 5-years period ending in December 1989 in children aged 4-14 years, Studied 51 children with a first attack of acute RF, carditis was in 43%,whileRF recurrences detected in 22 children, of them 91% hadcarditistherefore concluded that carditis was more frequent in patients with recurrent attacks. [10]A forth study was conducted in Riyadh, over a 10-year period (1994–2003), mean age of presentation of RF was 9 years, cardiac involvement was recognized in 44 patients(53%); of them, severe presentation was in 14 cases (32%). [11] The previous data supports that RF in Kingdom of Saudi Arabia is highly associated with cardiac involvement and cardiac tissue damage.

The autoimmune mechanisms underlying rheumatic carditis following streptococcal infection remain unknown. Noteworthy, half of children infected with GABHS strainshaddeveloped RHD. [12]in addition, RF appears to be a heritable condition: in 1889, familial predisposition was reported by Cheadle, who observed that patients with a positive family history of RF had five times risk to have RFmore than children without. [13]Furthermore,Ameta-analysis of 435 twins with acute RF confirmed that genetic factors play a major role in

the etiology of RF as the monozygotic twin concordance (44%) significantly exceeds the dizygotic twin concordance (12%). [14]These researches show up the magnitude of the genetic factors to occurrence of RF, RHD and may detect severity of the disease. [15-18]

The TNF-alpha–induced protein 3 (TNFAIP3) gene on chromosome 6q23 and encode protein A20, an inhibitor for nuclear factor kB (NF-kB) signaling pathways.[19] The 6q23 region has recently been associate with autoimmunity. However, this region contains no known gene transcript. The clearest candidate gene in this locus with known function is TNFAIP3 gene. TNFAIP3 response has been associated with anti-TNF therapy in autoimmune diseases patients. A tag single nucleotide polymorphisms (SNP), rs2230926, in TNFAIP3 was widely researched, and was linked tonumerousinflammatoryandautoimmune illnesses. [20]

In RHD, this SNP was associated with the disease in Chinese population. The minor C allele associated with reduced risk of the disease. [21]We hypothesized that an accumulation of damaging rare genetic variants in immunity genes may provoke an uncontrolled immune response to GABHS infection, which may lead to RF and fibrosis of the valves/RHD.

So, we conducted this study to evaluate the association between TNFAIP3-, rs2230926 allele, as a promoter and Saudi RHD patients

II. Materials and Methods

Study Population

The patients and control groups were described in our previous studies.[12]Briefly, three hundred and twenty nine individual were collected. The study was approved by the Maternity & Children Hospital ethics committee. The World Medical Association Declaration of Helsinki was followed. All participants signed a fully informed and written consent form approved by the ethics committee. Diagnosis was made according to the modified Jones criteria and confirmed by echocardiography. [22] Patients were sub-grouped according to echocardiographic findings, as previously published.[12]The control group was described previously.[12]

2.2 Genotyping of TNFAIP3 polymorphism

The DNA extraction and genotyping method we described previously.[12]Briefly, the TNFAIP3 polymorphismrs2230926 was genotyped using TaqManAllelic Discrimination Assay (Applied Biosystems, Foster City, CA; assay ID C_7701116_10) as described by the manufacturer.

2.3 Statistical analysis

SPSS version 17 (IBM Statistics, Chicago, IL) was used for statistical analysis. Data were checked for Hardy-Weinberg equilibrium (HWE). The unpaired Student's t-test and Fisher Exact test were used. Genotype and allele frequencies were determined by direct counting. Odds ratios and 95% confidence intervals were calculated. A p-value <0.05 was significant.

III. Results

The demographic characteristics and clinical details of the study groups are summarizedin Table 1. We collected data from 124 patients diagnosed as rheumatic disease and 205 healthy control subjects. There were no significant differences in age and sex between patients and controlgroups. Out of the ordinary concept, rheumatic heart disease was more in male than female (61 versus 31%). Carditis was the foremost frequent in the studied Saudi population (100%) with MVL in 56% and combined mitral and aortic valve lesions (44%). Rheumatic arthritis comes afterwards (53%) then chorea , skin rash and subcutaneous nodules follow.

When comparing genotypes (Table 2), it was observed that, (TT), (TC) and (CC) genotypes showed non-significant differences between both patients and control groups (P= 0.3). Putting (T) allele side by side to (C) allele, (T) allele was non-significantly higher compared with thecorresponding values for (T) allele the study groups (P value = 0.1). When weigh (TT +TC) against (CC) on attempt to detect allele carriage, non-significant difference was detected and the same was found for (CC + TC) versus (TT).

Detailed accounts of genotypes of patients with valvular lesions and control groups are reviewed inTable 3. (TT) was the most common genotype in MVL , CVL and in control groups (frequency was 0.49, 0.48 and 0.58; respectively) followed by (TC) genotype (frequency was 0.40, 0.41 and 0.33; in the same order) and lastly (CC) genotype (frequency was 0.11, 0.11 and 0.09; in the same arrangement). Non-significant differences among the 3 groups in genotypic expression were noticed.

(T) allele was non-significant increased frequency in MVL , CVL and control groups (0.69, 0.69 and 0.74; respectively versus corresponding values for (C) allele 0.51, 0.31 and 0.26; in the same order).

Aiming to identify allele carriage, (TT + TC) opposed to (CC), non significant differences among MVL , CVL and control groups were detected, then again on comparing (CC + TC) against (TT) in the 3 studied groups, non significant differences were considered.

IV. Discussion

RHD remains a neglected disease. [23]The current study explorestheimpact of genetic variation of tSNPs in theTNFAIP3 on RHD ina pilot case control setting. There was no association between RHD and the tag SNP rs2230926;this signifies that this region is not a potential contributor to the risk of RHD in Saudi population.

We collected data from 124 patients diagnosed as rheumatic disease and 205 healthy control subjects. Out of the ordinary concept; rheumatic heart disease was more in male than female (61% versus 31%). Our observation was in accordance with a study in Kuwait,over the period of five years (1984 through 1988), 557 cases of acute rheumatic fever were diagnosed; 291 (52%) were boys and 266 (48%) were girls.[24]However,thiswas in divergence with a study from Northern Territory of Australia, from 1997 to 2010, in which female was the predominant gender (males, 162 per 100 000; females, 228 per 100 000).[25]

Carditis was the foremost frequent in the studied Saudi population, western side, 100% had carditis; with MVL in 56% and combined mitral and aortic valve lesions (44%). Rheumatic arthritis comes afterwards (53%) then chorea (12%), skin rash (3%) and subcutaneous nodules (2%) follow.The predominance of cardiac affection is due collection of data from a cardiac center and therefore, most of the referred patients had cardiac affection which was associated with other major and/or minor criteria.

In a previous study performed between January 1980 and December 1984, in the Eastern Province of Saudi Arabia, The clinical manifestations included arthritis in 80%, which was more than carditis (60%), and chorea in 6.6%.[8]In another study performed in 1993 in KSA, arthritis was recorded in 76% of the cases, carditis in only 43%, and chorea in 8%. Carditis cases have; 81.8% had mitral regurgitation, 13.6% combined mitral and aortic regurgitation, and 4.6% aortic regurgitation.[26]

Another study conducted in Children's Hospital in Riyadh, over 10-year (1994–2003). Arthritis was present it 37% of the cases and cardiac disease was found in 53% cases. The involvement of the mitral valve alone occurred in 59% of the cases as mitral regurgitation, while both aortic and mitral valve regurgitation were present in 25% of the cases, and aortic valve regurgitation in 9% others.[11]

In Saudi Arabia, a study was conducted in December 1989, among children with a first episode of acute rheumatic fever. They found 76% had arthritis and 43% had carditis. [10]In the same duration period, another study was reported in Kuwait, the incidence of acute rheumatic fever was studied prospectively over a period of five years (1984 through 1988). The mean annual incidence in the study period was 2.9/100,000 children. There was a decline in the incidence from 3.7/ 100,000 in 1984 to 2.5/100,000 in 1988. 27% of children with acute rheumatic fever presented as recurrences in 1985; this also declined to 11% in 1988.[24] They did not comment on the number of patients who had carditis , arthritis or other features in their study.

In another non-arabian developing country, in western Ukraine; a retrospective study searched acute rheumatic fever in children,conducted from 2000 to 2013, Carditiswas reported in 84.7%, Polyarthritis in 54.1%, Chorea in 25.9%, isolated chorea in 10.6%, Subcutaneous nodules in 5.9% and Erythema marginatum was documented in 8.2%.[27]

In the Northern Territory of Australia, from 1997 to 2010, acute rheumatic fever in childrenincidence was 162 per 100000 in males and 228 per 100000 in females. After a first ARF diagnosis, 61% developed RHD within 10 years.[25]During mid-1980s there was an outbreak of acute rheumatic fever during in the USA.Children were from the middle and upper socioeconomic classes.[28] From the previous studies, we can observe that rheumatic fever and rheumatic carditisarenotonly related to the socioeconomic standard and development of the country[29] but have immune-mediated and genetic background. This enforced many researchers to study different genes to explore their impact on this immune mediated disease.

The mechanism linking the TNFAIP3 gene to theprevalence of RHD has to be explored, however, it is associated with increased risk of many immune mediated diseases as rheumatoid arthritis[30], systemic lupuserythematosus (SLE)among individuals of European ancestry[31],in Chinese Han population [32]and in the Japanese population[33],primary Sjogren'sSyndrome-associated Non-Hodgkin Lymphoma[34]andpsoriasis.[20]

In our study, it was observed that, (CC), (TC) and (TT) genotypes showed no significant differences between both patients with rheumatic fever and control groups. When weigh (TT +TC) against (CC)and for (CC + TC) versus (TT) as an attempt to detect allele carriage, no significant difference was detected.Indicating the lack of association between rheumatic fever with the tag SNP rs2230926.In addition, no specific allele carriage among MVL, CVL and control groups were detected. Indicating the lack of association between rheumatic carditis and rheumatic valvular affection with the tag SNP rs2230926.

In anotherstudy conducted in Chinese Han population, they investigated the association between TNFAIP3 polymorphisms(rs719149, rs5029935, rs582757), TRAF1 polymorphisms(rs10435843, rs2239657), (TRAF1) complement component 5 (C5) gene (rs12237774, rs4837805, rs17611, rs1017119, rs10818500),and rheumatic heart disease. However, they did not studytheimpact of genetic variation of tSNPs in theTNFAIP3rs2230926 on the RHD.[21]

Study limitations

This is a case control study with a relatively small sample size, which limited the statistical power of the study.

V. Conclusion

TNFAIP3 tSNP rs2230926 examined in this study has no role in predicting the occurrence and severity of RHD in Saudi patients. Further studies on larger number of patients in multicenter and multi-ethnic population are needed before universally apply these results.

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Table 1 Demographic characteristics and clinical details of the controls (*N*= 205) and patients (*N*= 124).

Parameter		Value
Average age, year (mean ± *SD* years):		
	Controls	20 ± 4
	Patients	19 ± 5
	Age of Onset	8 ± 2
Gender: Male/Female (%):		
	Controls	55/45
	Patients	61/39
Clinical manifestations of patients:		*Ncases* (%)
Characteristics		
	Valvular lesion	
	Mitral valve lesion (MVL)	70 (56)
	Combined valve lesion (CVL)	54 (44)
	Arthritis	66 (53)
	Chorea	15 (12)
	Skin rash	4 (3)
	Subcutaneous nodules	3 (2)
Laboratory findings at presentation		
	Elevated acute phase reactants (CRP/ESR)	88 (71)
	Prolonged PR interval	57 (46)

N: Number, *Ncases*: Number of cases, *SD*: Standard Deviation
CRP: C-Reactive Protein, *ESR:* Erythrocyte Sedimentation Rate

Table 2 Genotypes of the controls (*N*= 205) and patients (*N*= 124).

	Control (N= 205)		Patients (N= 124)					
Genotype	Count	Freq	Count	Freq	χ2	*df*	*p*-value	
TT	118	0.58	60	0.48	2.6	2	0.3	
TC	68	0.33	50	0.40				
CC	19	0.09	14	0.11				
Allele Freq					χ2	*df*	*p*-value	OR (95%CI)
T	304	0.74	170	0.69	2.4	1	0.1	0.8 (0.5-1.1)
C	106	0.26	78	0.31				
Allele Carriage								
(TT+TC) vs CC	186	0.91	110	0.89	0.35	1	0.6	0.8 (0.4-1.7)
(CC+TC) vsTT	87	0.42	64	0.52	2.6	1	0.1	1.5 (0.9-2.3)

Table 3 Genotypes of the controls (*N*= 205) and patients (*N*= 124).

Genotype	Control (N=205)		Patients (N=124)						
			MVL (N=70)		CVL (N=54)				
	Count	Frequency	Count	Frequency	Count	Frequency	*p*-value[1]	*p*-value[2]	*p*-value[3]
TT	118	0.58	34	0.49	26	0.48	0.4	0.4	0.9
TC	68	0.33	28	0.40	22	0.41			
CC	19	0.09	8	0.11	6	0.11			
T	304	0.74	96	0.69	74	0.69	0.2	0.3	0.8
C	106	0.26	44	0.51	34	0.31			
(TT+TC) vs CC	186	0.91	62	0.89	48	0.89	0.7	0.8	0.8
(CC+TC) vsTT	87	0.42	36	0.51	28	0.52	0.08	0.3	0.9

MVL: Mitral valve Lesion, CVL: Combined Valve Lesion, N: Number

[1]Analysis between controls and MVL subgroup

[2]Analysis between controls and CVL subgroup

[3]Analysis between MVL and CVL subgroups

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