

## Tumour necrosis factor (*TNF*)- $\alpha$ -308 gene polymorphism in Indian patients with Takayasu's arteritis - A pilot study

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**Background & objectives:** Tumour necrosis factor-alpha (*TNF*- $\alpha$ )- 308 promoter gene polymorphism has been shown to be associated with several autoimmune disorders and infections such as tuberculosis. There is no study on *TNF*- $\alpha$  gene polymorphism in Takayasu's arteritis (TA) till date. We aimed to study this polymorphism in TA, a granulomatous vasculitis, probably triggered by *Mycobacterium tuberculosis*.

**Methods:** *TNF*- $\alpha$  - 308 gene polymorphism was studied in 34 patients with TA and 39 healthy controls recruited from Christian Medical College, India. PCR was done followed by enzyme digestion. G and A polymorphisms were analysed. Occurrence of alleles in the disease group was compared with controls as well as with historical controls.

**Results:** GG allele was most frequent in TA and in controls. GA allele was detected in four controls but only in one patient who was the oldest in the study group. AA polymorphism was detected in one control but not in TA. When compared with controls from other populations, it was found that our allelic frequency was similar to that in Japan as well as from USA with mixed population. However, predominantly Caucasian population studied from Netherlands, Germany and England, where TA is rare, had a higher frequency of A allele as compared to our controls.

**Interpretation & conclusions:** Our preliminary results indicated that G allele at *TNF*- $\alpha$  - 308 was more common in TA patients and controls similar to that in other Indian as well as Japanese population. Compared to the western population, A allele was relatively less common in our study subjects.

**Key words** Giant cell arteritis - India - polymorphism - Takayasu's arteritis - *TNF*- $\alpha$  -308

Takayasu arteritis (TA) also known as pulseless disease, is an idiopathic chronic inflammatory disease of aorta and its branches leading to stenosis, occlusion and aneurysmal dilatation. In majority, the disease starts around 3<sup>rd</sup> and 4<sup>th</sup> decade of life. TA predominantly affects women with a female:male ratio of 5:1<sup>1</sup>. It is

most commonly seen in India, Japan, South East Asia and Mexico. Incidence in Indian population has not been calculated. It is believed to be similar to Japan where the reported incidence is nearly 150 per million per year. In western Europe and North America, it is only 0.2 to 2.6 per million<sup>2</sup>.

Tumour necrosis factor (TNF) is a cytokine with pleomorphic actions. TNF- $\alpha$  is pivotal in host defense against infections and has a major role in autoimmune diseases as well. It is also a crucial cytokine for granuloma formation. The level of TNF- $\alpha$  varies from individual to individual and is genetically determined<sup>3</sup>. The gene for TNF- $\alpha$  is located within the major histocompatibility complex (MHC) region on chromosome 6p21.3 which is a highly polymorphic region. There are many biallelic single nucleotide polymorphisms (SNPs) in and around the *TNF- $\alpha$*  gene. One such G/A polymorphism is located upstream of gene at -308 and is known to influence TNF- $\alpha$  levels. As compared with the - *TNF- $\alpha$*  -308G allele, A allele has higher transcriptional activity<sup>4,5</sup>.

*TNF- $\alpha$*  - 308 promoter gene polymorphism has been reported to be associated with several autoimmune disorders including systemic lupus erythematosus, rheumatoid arthritis and infections such as tuberculosis<sup>6</sup>. There was no study on *TNF- $\alpha$*  gene polymorphism in TA during the manuscript writing. During the manuscript review period, we came across one study reporting no TNF- $\alpha$  promoter polymorphism in TA amongst Han Chinese population<sup>7</sup>. We, therefore, carried out this pilot study to analyse TNF- $\alpha$  -308 polymorphism in TA which is a granulomatous vasculitis with an autoimmune basis, probably triggered by *Mycobacterium tuberculosis*<sup>8</sup>.

### Material & Methods

**Sample collection:** Consecutive patients with TA fulfilling American College of Rheumatology (ACR) 1990 criteria were recruited from outpatient and inpatient services of Clinical Immunology & Rheumatology and Cardiology Departments of Christian Medical College, Vellore, India, between October 2009 and May 2010. Matched healthy controls were also reported from amongst the hospital staff. After obtaining informed consent, 2 ml of peripheral blood was collected and stored at -20 °C till DNA was extracted. Study protocol was approved by the Institutional Review Board of Christian Medical College, Vellore.

**DNA extraction:** DNA was extracted using commercially available DNA extraction kit (QIAGEN) and stored at -20°C. Genotyping for *TNF- $\alpha$*  polymorphism was done for the isolated DNA for the polymorphic sites -308 by polymerase chain reaction (PCR) using thermal cycler (Applied Biosystems Gene Amp PCR System 9700, USA). Polymorphisms were analysed using restriction fragment length polymorphism (RFLP) method and primers specific to sites -308 was used. PCR was carried out in 25  $\mu$ l

reactions as previously described<sup>2</sup>. Forward primer 5'AGG CAA TAG GTT TTG AGG GCC AT 3' and reverse primer 5'TCC TCC CTG CTC CGA TTC 3' (Sigma, USA) were used to amplify a 107 bp fragment and then digested using *NcoI* restriction enzyme (New England Biolabs) and incubated at 37°C for 12 h. The analysis for the restriction fragments obtained (87 and 20 bp for -308G and 107 bp for -308A) was done on 2 per cent agarose gel. Each batch of samples was accompanied by positive and negative controls.

**Statistical analysis:** Frequencies of the genotypes between the study and the healthy groups were compared using Fisher's exact test. Results were also compared with historical controls from various populations across the globe.

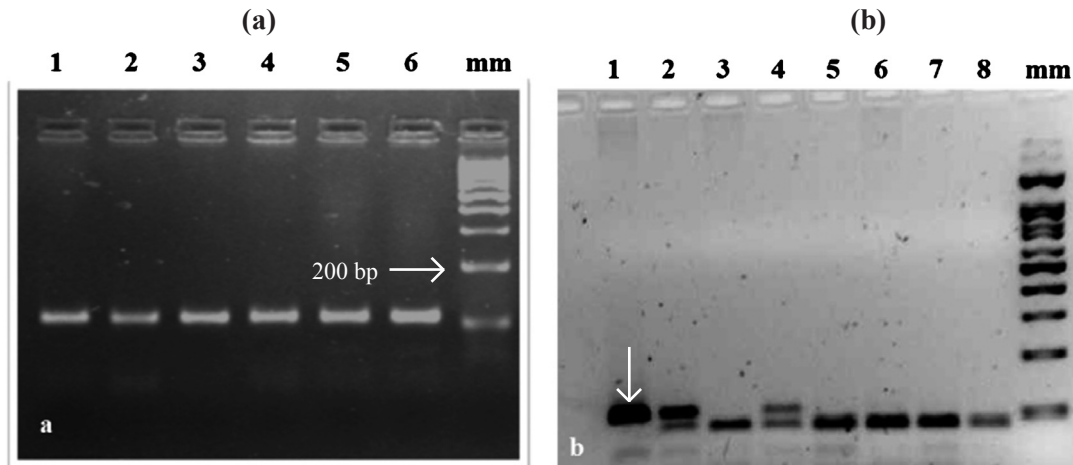
### Results & Discussion

A total of 33 patients with TA (28 female, 5 male) and 39 healthy controls were included in the study. The median age of onset of disease group was 27 yr (range 10-62 yr). Four (12.12 %) patients had disease onset at >50 yr. The median age of the controls was 25 yr (range 21-42). Female to male ratio were 28:5 for the patients and 29:10 for the control group respectively. The most common angiographic type was Type V that was seen in 18 patients (53%). Types I, III, II and IV were seen in 6, 5, 3 and 1 patient, respectively.

PCR for the *TNF- $\alpha$*  gene gave a 107-bp fragment (Fig. a). Following enzyme digestion of the PCR product, three results were obtained. A complete *NcoI* cut for homozygous *TNF- $\alpha$*  (-308G/G), resulting in two fragments of 87 and 20 base pairs; a partial cut for heterozygous *TNF- $\alpha$*  (-308G/A), resulting in three fragments of 107, 87 and 20 base pairs; and an uncut homozygous *TNF- $\alpha$*  (-308A/A), resulting in a 107 base pair fragment (Fig. b).

Genotype distribution is given in Table I. The difference in frequency of genotype distribution of the polymorphism between patients and controls was not statistically significant. However, no AA allele was detected in the disease group. GA allele was detected in the oldest patient with TA.

Allelic frequencies of this polymorphism in the control group were compared with that of different populations using Fisher's exact test. G allele was significantly more in our controls as compared to population of England ( $P=0.008$ ), Netherlands ( $P=0.002$ ), Germany ( $P=0.014$ ) but not significant when compared to the population of USA and Japan (Table II).



**Fig. (a)** Agarose gel (2%) electrophoresis showing PCR products of *TNF- $\alpha$*  gene promoter region after amplification. mm -100 bp marker; Lanes 5 1 to 6-107 bp PCR product. **(b)**. Agarose gel (2%) electrophoresis depicting restriction digestion of *TNF- $\alpha$* (-308 polymorphism) with *NcoI* enzyme Lane 1-undigested PCR product; Lanes 2 and 4-GA allele; 3, 5, 6, 7, 8- GG allele;mm -100 bp marker.

The allele frequencies of *TNF- $\alpha$*  polymorphism (-308) of cases and controls were not statistically different. The allelic frequency was found to be similar to that of an earlier study on Indian population<sup>9</sup>. It is noteworthy that in Japan and India where TA is commonly seen, the G allele frequency is higher than that of the other countries. In England, Netherlands and Germany, G allele frequency is relatively less and A

allele frequency relatively high as compared to India and Japan (Table II). There was no difference when our controls were compared with controls from USA; this apparent discrepancy may be because 40 per cent of their controls were of non-Caucasian ancestry. Therefore, perhaps G allele frequency closely mirrors the distribution of TA. It is well known fact that geographic distribution of Giant Cell Arteritis (GCA) is in sharp contrast to that of TA<sup>10</sup>. GCA is least common in Japanese and Asian Indians and is predominantly seen in the Scandinavian nations and North European countries<sup>10</sup>.

It has been shown in earlier studies that *TNF- $\alpha$*  -308 G allele is associated with lesser *TNF- $\alpha$*  production as compared to A allele<sup>4,5</sup>. Does GG allele predispose to TA and does AA allele protect from developing TA? Considering the fact that *TNF- $\alpha$*  is reportedly involved

**Table I.** Genotype distribution of alleles of *TNF- $\alpha$*  polymorphism TA patients and controls

Study group	Genotypes (%)		
	GG	GA	AA
TA (n=34)	33 (97.06%)	1 (2.94%)	0
Control (n=39)	34 (87.18%)	4 (10.26%)	1 (2.56%)

**Table II.** Allele frequencies of *TNF- $\alpha$*  polymorphism (-308) in different populations

Population studied	Genotype(percentage)			Frequency		<i>P</i> value*
	GG	GA	AA	G allele	A allele	
England <sup>11</sup>	96 (62.7)	52 (33.9)	5 (3.2)	0.797	0.202	0.008
USA <sup>12</sup>	212 (78)	55 (20)	6 (2)	0.877	0.122	0.345
Netherlands <sup>13</sup>	50 (57)	34 (34)	4 (2)	0.761	0.238	0.002
Germany <sup>14</sup>	143 (66.2)	63 (29.6)	10 (4.6)	0.810	0.190	0.014
Japan <sup>15</sup>	23 (95.8)	1 (4.1)	0 (0)	0.980	0.020	0.254
India <sup>16</sup>	165 (86.8)	24 (12.6)	1 (0.5)	0.932	0.068	0.807
Present study TA	33 (97.06)	1 (2.94)	0 (0)	0.985	0.015	0.122
Present study control	34 (87.18)	4 (10.26)	1 (2.56)	0.9231	0.0769	

\*Comparison of allelic frequencies between control group of present study and different population

in pathogenesis of TA<sup>16,17</sup>, it may seem surprising that lesser levels of TNF- $\alpha$  production are associated with the disease.

This seemingly paradoxical finding needs to be explained in the light of existent literature on pathogenetic mechanism in TA. Low levels of TNF- $\alpha$  and TNF- $\alpha$  blockade predispose to tuberculosis, which is implicated in pathogenesis of TA<sup>8</sup>. Is it possible that those with GG allele are less competent than those with the GA or AA allele in mounting an immune response against tuberculosis and eliminating it from the body. This could result in persistence of tuberculous antigen predisposing individuals with GG allele to autoimmunity. Could this ultimately lead to TA in presence of correct mix of other genetic and environmental factors?

Also, elderly age group is usually susceptible to GCA, the western counterpart of TA. GCA shares similarities in pathophysiology with TA and it is likely that these similarities are more common in the late-onset TA. In our study GA allele was present in one of the four late onset TA patients. This patient was the oldest in the study group. Does -308A allele protect from developing large vessel vasculitis in early life? Looking for this polymorphism in late onset TA and GCA may throw more light in this regard. Our study is limited by a small sample size. However, this is only a preliminary study and larger studies are required to determine the strength of association between TNF- $\alpha$  gene polymorphism, especially in late onset TA and GCA.

In conclusion, G allele at TNF- $\alpha$  - 308 was more common in TA patients and controls similar to that in other Indian as well as Japanese population. Compared to the western population, A allele was relatively less common in our population.

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