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RESEARCH LETTER

Association study of two interleukin-1 gene loci with essential hypertension in a Pakistani Pathan population

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An association study of *IL-1β* -511C/T and *IL-1 RN* 86 bp VNTR polymorphisms with essential hypertension was carried out in a sample population of 500 Pakistani Pathan subjects selected randomly, comprising groups of 235 subjects with hypertension and 265 controls. The distribution of both genotypes and alleles was not statistically different in cases and controls. In conclusion, *IL-1β* -511C/T and *IL-1 RN* 86 bp VNTR do not contribute to the aetiology of essential hypertension in the Pakistani Pathan population investigated here.

Essential hypertension (EHT) is a complex disorder resulting from interactions between environmental and genetic factors. Family and twin studies suggest that approximately one-third of the variance in blood pressure is attributable to genetic determinants.[1] Despite extensive efforts and the wide range of strategies employed, genetic variants of this complex phenotype still remain elusive. The use of candidate genes in case–control study designs to investigate complex disorders has become an increasingly preferred method owing to its cost-effectiveness and reasonably high success rates.

The role of immunological mediators is gaining increasing interest in the aetiology of cardiovascular phenotypes such as EHT. C-reactive protein (CRP) has been shown to be a significant predictor of the development of EHT.[2,3] Allele -511T in the promoter region of the human interleukin-1 β (IL-1β) gene was associated with higher blood pressure among Chinese hypertensive individuals.[4] Allele 2 of a VNTR (variable number of tandem repeats) in intron 2 of the interleukin-1 receptor antagonist (IL-1 RN) gene has been associated with single-vessel coronary artery disease in an English population[5] and with EHT in an Australian population.[6]

We investigated one single-nucleotide polymorphism (SNP) in *IL-1β* (-511C/T) and one VNTR in *IL-1RN* (86 bp VNTR) in a case–control study design to assess their putative associations with EHT in a Pakistani Pathan population. Pathans are a unique ethnicity in terms of their diet, language and sociocultural habits. This ethnicity is also characterized by high consanguinity rates as a result of its closed cultural and social systems. Genetic studies in such populations are thus less susceptible to the bias of 'population stratification'. The high degree of consanguinity observed in Pathans is hypothesized to be responsible for the pooling of recessive alleles resulting in the overexpression of certain diseases. These include recessive monogenic diseases as well as complex diseases, for which increasing evidence suggests a mode of recessive inheritance of underlying major gene effects. This may explain the 56% prevalence rate of EHT in the Pathan ethnicity, which is twofold higher than in other Pakistani populations.[7]

The study was approved by the Ethical Review Committee of Aga Khan University. The sample size was calculated to detect an association at 80% power with an α of 5%. Subjects were recruited from the *Pathan Colony*, Karachi (an area of the city inhabited almost exclusively by Pathans who have migrated from the *North-Western Frontier Province* of Pakistan), through a two-stage selection process. The population census was used to randomly select households. This was followed by another census of these selected households. A total of 500 individuals over 40 years of age with almost equal gender distribution were randomly selected in the second stage. The two-stage random selection aimed at selecting equal proportions of normotensive (NT) and EHT Pathans.

A trained team, including a doctor and community health workers, carried out the recruitment of selected subjects. EHT was defined as systolic blood pressure (SBP) $\geqslant$140 mm Hg or diastolic blood pressure (DBP) $\geqslant$90 mm Hg, in supine position, after 20 min of rest on 2 separate days, or if the person was taking antihypertensive treatment. The mean of three readings was used in the analyses. To rule out any secondary cause of hypertension, complete medical history was obtained and physical examination was conducted on all subjects after they gave their consent. A pre-tested and coded questionnaire was used to record life style and risk factor data. NT subjects had no personal history of EHT (documented as resting SBPs $<$140 mm Hg and DBPs $<$90 mm Hg on at least three separate occasions) and no family history of EHT in direct relatives (parents and siblings). We remain aware of the fact that NTs in such studies may not be proper 'controls', as they are indeed simply free of disease at the time of study, but may develop

Table 1 Distribution of genotypes and allele frequencies among HT and NT Pathan subjects

	Total (500) N (rel. freq)	*NT (265) N (rel. freq)*	*HT (235) N (rel. freq)*	*Univariate OR (95% CI)*	P-*value*
*IL-1*β *-511 C/T*					
Genotypes					
CC	193 (0.39)	103 (0.39)	90 (0.38)	1.00	
CT	243 (0.49)	130 (0.49)	113 (0.48)	0.90 (0.52-1.56)	0.722
TT	63 (0.12)	32 (0.12)	31 (0.14)	0.90 (0.51-1.59)	0.702
Alleles					
C	629 (0.63)	336 (0.63)	293 (0.62)	1.00	
T	369 (0.37)	194 (0.37)	175 (0.38)	0.97 (0.75-1.25)	0.797
IL-1 RN 86bp VNTR					
Genotypes					
AxAx	234 (0.47)	115 (0.44)	119 (0.51)	1.00	
A2Ax	203 (0.41)	112 (0.43)	91 (0.39)	0.79 (0.54-1.15)	0.209
A2A2	60 (0.12)	32 (0.13)	24 (0.10)	0.64 (0.36-1.15)	0.135
Alleles					
Ax	671 (0.67)	342 (0.65)	329 (0.70)	1.00	
A2	323 (0.33)	184 (0.35)	139 (0.30)	0.79 (0.60-1.03)	0.076

Abbreviations: CI, confidence interval; HT, hypertensive; IL, interleukin; NT, normotensive; OR, odds ratio; rel. freq, relative frequency; VNTR, variable number of tandem repeats.

hypertension at a later stage. This is why a better term to characterize disease-free subjects would be a 'comparison' (rather than 'control') group.

A total of 10 ml of venous blood was taken in two different tubes for DNA extraction and serum analyses of creatinine, glucose and lipid profile. DNA was extracted from 5 ml venous blood using a standard phenol–chloroform protocol. Genotyping was carried out by PCR and restriction fragment length polymorphism (RFLP) techniques. The *IL-1β* -511C/T genotype was identified by PCR followed by RFLP using the restriction enzyme *Ava*I to digest the mutant allele as described previously.[5] *IL-1 RN* VNTR genotypes were identified by PCR as reported previously.[8]

Data were coded and entered in SPSS Windows (13.0). Mean values $\pm$ standard deviation (SD) were calculated for all variables. Alleles and genotype frequencies were determined by gene counting. In univariate analysis, categorical variables were compared using χ^2 tests. A value of $P<0.05$ was considered statistically significant.

This study included 500 Pathan subjects (265 NTs and 235 EHTs) with a mean age of 51.5 ± 10.1 years. Genotypes of both cases and controls were in Hardy–Weinberg equilibrium for *IL-1β* -511C/T polymorphism. For *IL-1 RN* 86 bp VNTR polymorphism, the genotypes were in Hardy–Weinberg equilibrium after converting it into a bi-allelic marker by merging all alleles other than A2 into a single allele (Ax). Allele frequencies for *IL-1β* -511C/T polymorphism in the overall population were 63.0% for -511C and 37.0% for -511T. For *IL-1 RN* VNTR polymorphism, allelic frequencies were 64.6% for A1, 32.5% for A2, 2.1% for A3 and 0.8% for A4. No A5 allele was found in this population. The allelic frequencies on both polymorphisms in our population were similar to those reported previously in some Caucasian populations.[6–8] Table 1 shows the distribution of genotype and allele frequencies for *IL-1β* -511C/T and *IL-1 RN* VNTR polymorphisms in NT and EHT groups. No significant association was observed upon comparison of either genotype distribution or allele frequencies between EHTs and NTs.

Our aim was to make use of the relative genetic homogeneity of our population to explore putative associations of two cytokine gene polymorphisms with EHT. Investigating the Pathan consanguineous ethnic group minimizes the influence of both selection bias and population stratification.

The role of immunological reactivity in the aetiology of cardiovascular pathology is gaining increasing interest. With the recognition that atherosclerosis is an inflammatory process,[9,10] the attention is now focused on the role of inflammatory pathways in the aetiology of cardiovascular diseases, including EHT. In a cohort of 20 525 subjects with no history of cardiovascular events or malignancies, CRP was found to be a significant predictor of developing hypertension independently of other coronary risk factors.[2,3] These results strongly implicate that the aetiology of hypertension has a significant inflammatory basis. Some investigators believe that inflammation within the arterial tree owing to the effects of atherogenic lipoproteins, low high-density lipoprotein, high plasminogen activator inhibitor-1, insulin resistance and smoking, releases cytokines that increase CRP levels.[3,11] Another explanation for this phenomenon is that CRP may play a direct role in the causation of hypertension through its proinflammatory influence.[2,3] In either case, cytokines seem to play an essential role in this pathway.

In this study, we investigated the association between two cytokine gene polymorphisms (*IL-1β* -511C/T

and *IL-1 RN* VNTR) and EHT. None of the studied genetic markers showed statistically significant differences in the distribution of genotypes or alleles among NT and EHT groups. Moreover, none of the polymorphisms was associated with diabetes, smoking status or body mass index. As a number of EHT subjects were on blood pressure lowering medications, association analyses of genetic markers with blood pressure levels could not be performed.

The results of this study are different from those reported in some other populations.[4,6] A possible reason could be that these genes may have a minor role in the causation of EHT; to evidence such effects, larger sample sizes would be needed. Alternatively, this genetic effect could be population-specific only. Moreover, the SNPs investigated here may not sufficiently cover the functional haplotype in all the ethnicities, and thus thorough investigations on linkage disequilibria among different variants within these genes should be performed in further association studies. To gain more insight into the disease pathogenesis, circulating levels of inflammatory markers will also need to be assessed in study participants.

In conclusion, our study does not support the relevance of the *IL-1β* -511C/T and *IL-1 RN* VNTR polymorphisms in the aetiology of EHT among Pakistani Pathans. More studies, however, are needed to study the role of other variants in genes of these markers and other cytokines to decipher the complex immunologic pathways contributing to the aetiology of EHT.

What is known about the topic

- After the recognition of atherosclerosis as an inflammatory process, role of inflammatory pathways in the aetiology of hypertension is increasingly being deciphered
- *IL-1β* and *IL-1 RN* polymorphisms have been reported to be associated with variation in blood pressure

What this study adds

- *IL-1β* -511C/T and *IL-1 RN* variable number of tandem repeats polymorphisms are not associated with essential hypertension in a Pakistani Pathan population

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