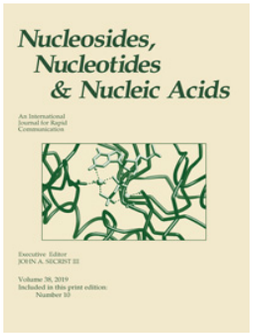

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The effect of tumor necrosis factor alpha (-308G/a) and interferon gamma (+874T/a) polymorphisms on susceptibility to coronary heart disease

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ABSTRACT

Background: Coronary heart disease (CHD) is a chronic inflammatory disease, which is still regarded as a major cause of morbidity and mortality worldwide. Several studies have suggested that polymorphisms in cytokine genes are associated with the pathogenesis of CHD. The genotype distribution of Tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) genes polymorphisms have been shown to be different in various ethnic populations. This study was aimed to investigate the association of TNF-α-308 G/A and IFN-γ + 874T/A polymorphisms with risk of CHD in an Iranian population.

Methods: A total of 187 unrelated subjects comprised 96 CHD patients and 91 healthy controls were enrolled in this cross-sectional study. The TNF-α-308 G/A and IFN-γ + 874T/A polymorphisms were genotyped using amplification refractory mutation system-PCR (ARMS-PCR). The chi-square and logistic regression tests were used to calculate the odds ratios (ORs) as a measure of differences in genotype frequencies.

Results: A significant differences in the allelic and genotypic distribution of TNF-α-308 G/A and IFN-γ + 874T/A polymorphisms was found between CHD patients and healthy controls ($P = 0.017$, $P = 0.011$, $P = 0.006$ and $P = 0.002$, respectively). Logistic regression analyses were also revealed statistically significant risk for CHD with respect to TNF-α-308 A and IFN-γ + 874 T carriers either in crude or after adjustment for potential confounders ($P = 0.003$ and $P = 0.006$, respectively).

Conclusion: This study provides strong evidence supporting the association of TNF-α-308G/A and IFN-γ + 874T/A polymorphisms with the increased risk of CHD. Therefore, these two cytokine polymorphisms may play a role in predisposition to coronary heart disease.

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Introduction

Coronary heart disease (CHD) is a chronic inflammatory condition, which is considered as an outstanding health problem and the cause of the epidemic proportions of premature morbidity and mortality worldwide.[1] This multifactorial disease result from a complex interaction between genetic susceptibility and environmental lifestyle factors such as diet and physical inactivity.[2,3] Various clinical manifestations such as sudden cardiac death, thromboembolic events, stable angina and acute coronary syndrome are often shown to be associated with patients affected by coronary heart diseases.[4] It was also proposed that abnormal function of immune system resulted from abnormalities in inflammatory mediators is also involved in CHD development. Cardiovascular risk factors including; premature atherosclerosis, coronary artery disease, dyslipidemia, diabetic retinopathy, hypertension, endothelial dysfunction, and clotting activation, are all associated with alteration in immune system.[5] It is known that this syndrome is a disorder of the immune system, in which, various aberrations such as increased production of pro-inflammatory cytokines could be happened.[6]

Many research works have been conducted to identify the genes underlying susceptibility to CHD and replicate significant single nucleotide polymorphisms (SNPs) in different populations. Based on the data from previous studies, SNPs within the cytokines genes have been linked to chronic inflammatory situation.[7] Recent studies have investigated the association between genetic mutations in two kind of cytokines; Tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) and susceptibility to CHD.[8] These cytokines have a crucial role in immune system imbalance in CHD and some other inflammatory disorders.[9,10] TNF-α gene is located on the human chromosome 6 (ch.6p21.3) in close linkage with MHC class III (major histocompatibility complex) genes.[11] This cytokine is produced by macrophages, monocytes, neutrophils, as well as T and B cells and regulated by other anti-inflammatory cytokines like Interleukin 10 (IL-10).[12] Hitherto, eight diverse polymorphisms of this gene have been distinguished. Polymorphism at position $-308\,G > A$ is one of the most interesting investigated SNPs. Substitution of G to A nucleotide leads to a decrease in TNF-α expression which is correlated with CHD processes and outbreak.[13,14] The human IFN-γ, also known as type II interferon or macrophage-activating factor (MAF), is located on chromosome 12 (ch12q24.1).[15] IFN-γ is a potent inflammatory mediator, playing the crucial role in autoimmune disorders and chronic inflammatory diseases. IFN-γ gene variants seem to be extremely important in conferring susceptibility to CHD. One of the notable polymorphisms at position $+874\,T > A$

has a significant function in the propagation of CHD. In particular, T allele at +874 situation enhanced the expression of IFN-γ gene.[16]

A growing body of evidence have proposed a possible association between TNF-α-308 G/A and IFN-γ + 874T/A polymorphisms and susceptibility to CHD. Some studies have found a direct correlation between these polymorphic variants and predisposition to CHD, while the others showed contradictory results and some studies have failed to find any association [7,9 &15]. Besides, increased level of IFN-γ and TNF-α cytokines were shown to be associated with disease activity in CHD patients. Existing data suggest that inflammatory markers may have a crucial role in immune system imbalance in CHD and some other inflammatory diseases [1, 2, & 5]. With regard to the difference in genotype distribution of TNF-α and IFN-γ genes polymorphisms within various ethnic populations, this study was aimed to investigate the association of TNFα-308 and IFNγ + 874 polymorphisms with the risks of CHD in the north center of Iran.

Methods

Study populations

A total of 187 genetically unrelated Iranian subjects comprised 96 CHD patients and 91 healthy controls were enrolled in this cross-sectional study. All study participants consecutively recruited from hospitals located in Damghan city in the north center of Iran. Indication for CHD was on the base of a set of examination tests including, a complete clinical examination, an electrocardiogram (ECG) test at rest and a routine angiography if appropriate.[17] Healthy individuals with no history of CHD who had undergone regular physical examinations during the same time were also included as control group. All subjects with rheumatic heart disease, congenital heart disease and other comorbidity such as malignancy, chronic infections, chronic renal failure, and pregnant women were excluded from the study. Potential controls were also excluded if they had a family history of CHD, diabetes, hypertension, or were taking any kind of medications for hypertension, diabetes and lipid or glucose metabolism.

All participants were given a standardized questionnaire to record all required information. These information included the age, sex, ethnicity, details of medical history, family history of CHD, diabetic status and other traditional risk factors of CHD such as cigarette smoking, physical activity, along with physiological and anthropometric data. Written informed consent was obtained from study participants and the study was carried out in accordance with declaration of Helsinki and approved by the local ethical committee at University of Damghan.

The laboratory measurement of inflammatory markers

Serum level of two studied inflammatory markers in patients with CHD and healthy controls were measured using commercially available enzyme linked immunosorbent assay kits (ELISA, Diaclone, France). All measurements were performed according the manufacturer's instructions on epoch microplate reader as ELISA reader instrument.

DNA isolation, SNP selection and genotyping

Peripheral blood was sampled, and genomic DNA was extracted using a commercial kit according to the manufacturer‘s instruction (5 prime, Germany).

Two common SNPs including TNF-α-308G > A and the INF-γ + 874 T > A with minor allele frequency (MAF) ≥ 0.05 were selected based on previous publications.[1,18–22] Genotyping of TNF-α-308G > A and INF-γ + 874T > A polymorphisms was performed by polymerase chain reaction (PCR) based on amplification-refractory mutation system (ARMS) analysis. Generic primers and PCR conditions for TNF-α-308G > A were: sense primer G: 5′-ATAGGTTTT GAGGGGCATGG sense primer A: 5′-AATGGTT TTGAGGGGCATGA-3′ and antisense primer 5′-TCTCGGTTTCTTCTCC ATCG-3′. Conditions for INF-γ + 874 T > A were: sense primer T: 5′-TTCTTACAACACAAAATCAAATCT-3′, sense primer A: 5′-TTCTTACAA CACAAAATCAAATCA-3′ and antisense primer: 5′-TCAACAAAGCT GATACTCCA-3′. The amplification conditions for the ARMS-PCR were of denaturation at 94 °C for 20 s, annealing at 63 °C for 1 min for TNF-α and 62 °C for 55 s for IFN-γ, DNA extension at 72 °C for 60 s and the final extension at 77 °C for 3.20 min. Afterward, PCR products were checked on a 2% agarose gel to detect the expected length of 184-bp for (-308G > A) and 263 bp for (+874 T > A) sites (Figures 1 and 2). For the genotyping quality control, 10% of samples were randomly selected and measured in duplicates and the concordance rate was 100%. Nuclease-free water was used as negative control.

Statistical analysis

Data were analyzed using SPSS software version 16 (Inc., Chicago, Il, USA). The allelic frequency and distribution of genotypes in cases and controls, the association between genotypes and clinical groups and analysis of deviation from the Hardy–Weinberg equilibrium were assessed using Chi-square test. The quantitative variables were expressed as mean with standard deviations. Multiple logistic regression analysis were used to calculate odds ratios (OR) with 95% confidence intervals (CI) either in crude or

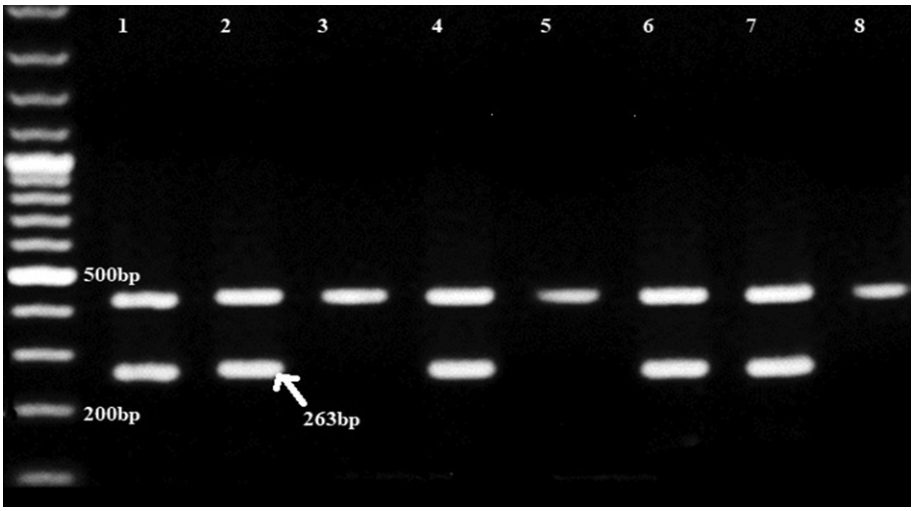

Figure 1. ARMS-PCR of the INF-$\gamma + 874$ A/T gene polymorphism of different individuals based on polyacrylamide gel electrophoresis 2% stained with ethidium bromide. Left lane: 100 bp DNA ladder. Lane 1: sample without DNA. Lane 2: sample plus Beta-actin. Lanes 1 and 2: We used both primers which revealed the A and T alleles. So, this pattern belongs to an individual who has AT heterozygote genotype. Lanes 3&4: We applied a primer which showed the A allele. So, they are homozygote for AA genotype, the same as lane 5&6. Lanes 7 and 8: we used a primer which showed the T allele. So, they are homozygote for TT genotype. Both alleles have the same molecular weight.

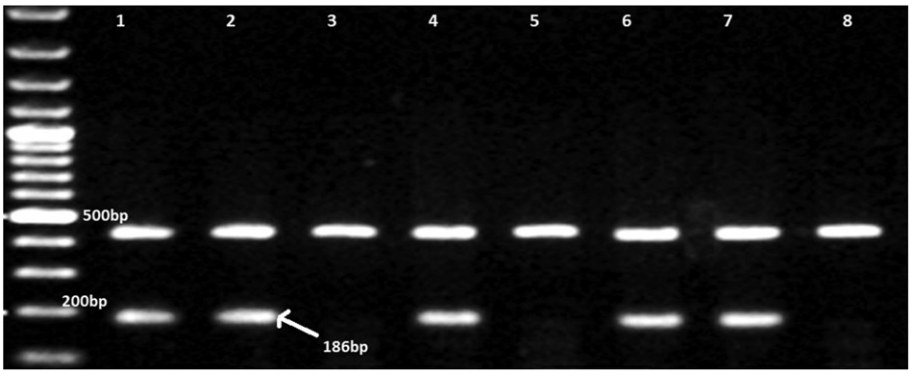

Figure 2. ARMS-PCR of the TNF-α-308 G/A gene polymorphism of different individuals based on polyacrylamide gel electrophoresis 2% stained with ethidium bromide. Left lane: 100 bp DNA ladder. Lanes 1 and 2: We used both primers which revealed the G and A alleles. So, this pattern belongs to an individual who has GA heterozygote genotype. Lanes 3&4: We applied a primer which showed the G allele. So, they are homozygote for GG genotype, the same as lane 5&6. Lanes 7 and 8: we used a primer which showed the A allele. So, they are homozygote for AA genotype. Both alleles have the same molecular weight.

adjusted for probable confounders, at codominant genetic model. Bonferroni correction was also applied for the allelic tests and the threshold for significant association (0.05/2 tested SNPs), was set to $P \leq 0.025$.

Results

The basic characteristics of study subjects are listed in Table 1. The study included 96 CHD patients (66 men and 30 women; mean age, 48.39 ± 14.26 years) and 91 healthy controls (49 men and 42 women; mean age, 45.32 ± 11.35 years). As expected, the traditional risk factors including smoking (61.45%), hyperlipidemia (63.51%), hypertension (51.04%), diabetes mellitus (68.75%) and hypercholesterolemia (46.87%) were found to be higher in the patients with CHD than the controls.

The allele and genotype frequency differences of two analyzed SNPs between case and control groups are shown in Table 2. All two SNPs were in Hardy-Weinberg equilibrium (HWE) in the controls ($p > 0.05$). However, CHD cases showed a significant deviation from HWE with respect to the TNFα-308G > A and INF-γ + 874 T > A polymorphisms. These deviations may be due to the small sample size. However, it could not be a reflection of violation of HWE assumptions in the general population, and such a departure can be attributed to the chance. Using chi-square tests, our results determined the A allele of TNFα-308 and T allele of INF-γ + 874 were associated with a 1.81-fold (95%CI = 1.11-2.96; $p = 0.017$) and 2.06-fold (95%CI = 1.30-3.26; $p = 0.006$) increase in the risk of CHD respectively (Table 2).

Regard to the genotype frequencies, our results were also revealed a significant difference in genotype distributions of two SNPs between patients and healthy subjects ($p = 0.011$ for TNF-α-308 and $p = 0.002$ for INF-γ + 874). Logistic regression analysis indicated that compared to the GG genotype of TNF-α-308 polymorphism, subjects carrying the A allele (AA + GA) were significantly correlated with an increased risk of CHD either in crude (OR = 2.40, (1.33–4.35), $p = 0.011$) or even after multiple-testing correction for two SNPs and adjustment for age, sex, and other potential confounding factors (OR = 3.10, (1.49–6.48), $p = 0.003$). Similarly,

Table 1. Demographic and clinical characteristics of the study population.

Characteristics	CHD patients (n = 96)	Controls (n = 91)	*p*-value
Age (years)	48.39 ± 14.26	45.32 ± 11.35	0.004
Gender (M/F)	66/30 (68.75/31.25)	49/42 (53.84/46.15)	0.02
Smoking n (%)	59 (61.45)	39 (42.85)	0.01
Diabetes mellitus n (%)	66 (68.75)	42 (46.15)	0.002
Hyperlipidaemia n (%)	61 (63.51)	37 (40.65)	0.002
Hypertension n (%)	49 (51.04)	29 (31.86)	0.008
Sedentary lifestyle n (%)	43 (44.8)	36 (39.56)	0.46
Obesity n (%)	40 (41.70)	37 (37.4)	0.547
TC (mg/dl) n (%)	45 (46.87)	28 (30.76)	0.02
IFN-γ	0.38 (0.00–0.57)	0.57 (0.00–0.73)	0.021
TNF-α	1.60 (1.23–1.90)	1.80 (1.21–2.40)	0.027

Values are expressed as mean ± SD, median and interquartile range for normally and non-normally distributed variables, respectively. Comparisons were made by the X^2 test (for categorical data). TC; total cholesterol.

more than 2.5 fold increased risk was observed for all the genotypes of IFN-γ + 874 A/T, either in crude or after adjustment for confounding variables (Table 2). In addition, after applying Bonferroni's correction no considerable changes were also observed for the results, supporting no interaction between two study variants.

We also analyzed the association of the different genotype groups of each polymorphism with all above mentioned traditional risk factors. The results are presented in Table 3. As can be seen from Table 3, IFN-γ + 874

Table 2. Genotype distribution and allele frequencies of the TNFα-308 and INF-γ + 874 polymorphisms in CHD patients and controls.

Polymorphism	CHD n (%) n = 96	Control n (%) n = 91	Crude OR (95 % CI)	Adjusted OR (95 % CI)	*P*-value[a]
TNFα-308					
GG	44 (45.80)	61 (67.03)	1.00	1.00	–
GA	49 (51.0)	27 (29.67)	2.52 (1.37-4.63)	3.56 (1.66-7.68)	**0.001**
AA	3 (3.10)	3 (3.30)	1.39 (0.27-7.19)	0.650 (0.086-4.94)	0.679
GA + AA	52 (54.16)	30 (32.96)	2.4 (1.33-4.35)	3.10 (1.49-6.48)	**0.003**
P[b] = 0.011					
G allele	137 (71.34)	149 (81.86)	1.00	1.00	–
A allele	55 (28.66)	33 (18.13)	1.81 (1.11-2.96)	1.81 (1.11-2.96)	**0.017**
INF-γ + 874					
AA	33 (34.37)	53 (58.24)	1.00	1.00	
AT	57 (59.37)	37 (40.65)	2.47 (1.36-4.51)	2.26 (1.09-4.69)	**0.028**
TT	6 (6.25)	1 (1.09)	9.63 (1.11-83.65)	46.47 (4.2-514.3)	**0.002**
AT + TT	63 (65.62)	38 (41.76)	2.66 (1.47-4.82)	2.70 (1.34-5.47)	**0.006**
P[b] = 0.002					
A allele	123 (64.06)	143 (78.57)	1.00	1.00	–
T allele	69 (35.40)	39 (21.42)	2.06 (1.30-3.26)	2.06 (1.30-3.26)	**0.006**

Multiple testing using logistic regressions was used. OR, odds ratio; CI, confidence interval. Significant *P* values are presented in bold.

[a]P of logistic regression models adjusted for age, sex, smoking, hypertension, hyperlipidemia, TC, physical activity, diabetes and hyperlipidemia.

[b]P of X^2 test related to allelic and genotypic differences between cases and controls.

Table 3. Association between TNFα-308 and INF-γ + 874 polymorphisms with respect to different risk groups.

		TNFα-308				INF-γ + 874			
		Genotype/allele							
		GG	GA	AA	p- value	AA	AT	TT	p- value
Smoking	Yes	55 (56.1)	39 (39.8)	4 (4.1)	0.769	36 (36.7)	58 (59.2)	4 (4.1)	**0.028**
	No	50 (56.2)	37 (41.6)	2 (2.2)		50 (56.2)	36 (40.4)	3 (3.4)	
Hypertension	Yes	49 (62.8)	27 (34.6)	2 (2.6)	0.29	31 (39.7)	44 (56.4)	3 (3.8)	0.34
	No	56 (51.4)	49 (45.0)	4 (3.7)		55 (50.5)	50 (45.9)	4 (3.7)	
Hyperlipidemia	Yes	51 (52.0)	44 (44.9)	3 (3.1)	0.46	45 (45.9)	51 (52.0)	2 (2.0)	0.42
	No	54 (60.7)	32 (36.0)	3 (3.4)		41 (46.1)	43 (48.3)	5 (5.6)	
Physical activity	Yes	41 (51.9)	36 (45.6)	2 (2.5)	0.48	38 (48.1)	38 (48.1)	3 (3.8)	0.87
	No	64 (59.3)	40 (37.0)	4 (3.7)		48 (44.4)	56 (51.9)	4 (3.7)	
Diabetes	Yes	59 (54.6)	43 (39.8)	6 (5.6)	0.10	51 (47.2)	55 (50.9)	2 (1.9)	0.27
	No	46 (58.2)	33 (41.8)	**0**		35 (44.3)	39 (49.4)	5 (6.3)	
Hypercholesterolemia	Yes	40 (54.8)	31 (42.5)	2 (2.7)	0.90	32 (43.8)	40 (54.8)	1 (1.4)	0.30
	No	65 (57.0)	45 (39.5)	4 (3.5)		54 (47.4)	54 (47.4)	6 (5.3)	
Obesity	Yes	45 (60.8)	26 (35.1)	3 (4.1)	0.34	37 (50.0)	33 (44.6)	4 (5.4)	0.34
	No	60 (53.1)	50 (44.2)	3 (2.7)		49 (43.4)	61 (54.0)	3 (2.7)	

Values are expressed as number (%). Both adjusted for age, and sex.

SNP showed a trend towards association with increased risk of CHD in cigarette smokers ($P = 0.028$). That is, SNP-smoking interaction may contributed in predisposing cigarette smokers to develop CHD.

Discussion

CHD is the first outstanding cause of mortality in adults all over the world. Various factors play a crucial role in the pathogenesis of CHD.[2] Environmental and genetic components are involved in the susceptibility of CHD.[7] Among traditional factors, diabetes mellitus has been strongly associated with CHD.[21] As expected, in our study, all traditional factors had the higher frequency (diabetes mellitus with a frequency of 68.75% as the highest and obesity with 14.58% as the lowest) in CHD patients than controls. Our results are in agreement with those reported by Ebrahimi et al. which concluded that the prevalence of CHD in the Iranian population was higher due to their lifestyle associated with coronary risk factors such as diabetes mellitus, hypertension, dyslipidemia, smoking and obesity.[22] Sadeghi et al. was also reported that traditional factors were associated with the prevalence of premature CHD.[23]

In addition to traditional cardiovascular disease risk factors, many genetic variants in genes encoding inflammatory cytokines such as TNFα and IFN-γ, were identified to play a role in susceptibility to CHD.[22] Two SNPs including IFN-γ + 874 A > T (rs2430561) and TNF-α-308 G > A (rs1800629), are defined as ideal candidate gene variants.[22,23] This study was conducted to investigate the possible association between these polymorphisms and risk of CHD in an Iranian population.

Our study demonstrated that allelic and genotypic distribution of TNF-α-308 G/A and IFN-γ + 874T/A polymorphisms were significantly different between patients and control subjects. With respect to TNF-α-308 G/A polymorphism logistic regression analysis confirmed that A carriers' (GA-AA vs GG) were strongly associated with increased risk of CHD in comparison with the GG homozygote group, suggesting a significant role of A allele in the development of CHD. Likewise, the genotype distribution of IFN-γ + 874T/A polymorphism was significantly associated with increased risk of CHD in all subject carrying T allele. Logistic regression analyses, also ascertained this findings, indicating more than 2.5 fold significant risk associated with this polymorphism, even after adjusting for all potential confounders. This suggests that the T allele may have a direct role in the pathogenesis of CHD. When these two polymorphisms were considered together, the observed associations continued to exist, suggesting the independent behavior of these two polymorphisms in the development of CHD.

We also evaluated the association of these two polymorphisms with traditional risk factors of CHD including hypertension, hyperlipidemia, hypercholesterolemia, smoking, diabetes mellitus, sedentary lifestyle and obesity. Our results indicated that the IFN-γ + 874T/A polymorphism was strongly associated with the risk of CHD in cigarette smokers, especially when the T allele interacts with cigarette smoking. This suggest that, SNP-smoking interaction may contributed in predisposing cigarette smokers to develop CHD.

In line with our results, the meta-results of a meta-analysis of 24 studies conducted by Zhang et al. revealed that TNF-α-308 polymorphism was associated with CHD development in Caucasians population.[3] This study supported the fact that the TNFα-308A allele may have a direct role in the pathogenesis of CHD. In another study by Naggar and Serogy, it was also reported that the TNF-α-308G/A polymorphism might be a potent risk factor for CHD.[24] In a meta-analysis on 5 studies, Yang et al. concluded that the TNF-α-308 A allele is probably associated with an increased risk of IHD.[12] The same results were also observed by George et al. in Greek familial CHD patients.[11] On the other hand, some reports were contrary to the results of our study. Garg et al. concluded that TNF-α-308 G/A polymorphism was not significantly different in CHD patients.[8] Chu et al. in a large sample size meta-analysis showed that no association existed between the TNF-α-308 promoter polymorphism and CHD in Chinese Han population.[25] This disparity between observations may be due to ethnic differences.

In terms of IFN-γ + 874T/A polymorphism, Baddela et al. showed that the IFN-γ + 874 risk allele was an important risk factor for the development of susceptibility to acute coronary syndrome.[7] Esperança et al. in their study demonstrated a significant relation between IFN-γ + 874T/A genotypic distribution and the prevalence of heart disease in young adults.[26] Our study confirms the previous findings. Many studies suggested INF-γ as an important component in regulating inflammation. Functional studies have found that T allele is significantly correlated with the increase of the INF-γ gene activity.[15]

The association of TNF-α-308 G/A polymorphism with the risk of CHD could be explained by its possible effect on the expression level of the TNF-α gene. It was proposed that most variants within the promoter region of the TNF-α gene appeared to influence the TNFα cytokine expression.[27] Likewise, IFN-γ + 874T/A polymorphism was also proven to cause an overexpression of the gene, where T allele may results in an increased production of IFN-γ cytokine.[19]

To our knowledge, this is the first report on the association of -308 G > A (rs1800629) and +874 A > T (rs2430561) SNPs with CHD. Both TNF-α-308A and IFN-γ + 874T alleles are important risk factors for the development of CHD in an Iranian population. Because, the study

population was originated from north center of Iran, it is proposed that the data should be cautiously extrapolated to other regions and required to be validated by further studies in other regions and ethnic groups

Conclusion

In conclusion, our results suggest that TNF-α-308 G/A and IFN-γ + 874 T/A polymorphisms are strongly associated with the increased risk of CHD. Furthermore, TNF-α − 308 A allele and IFN-γ + 874 T allele may be potent risk factors for CHD in the Iranian population. This threat is increased when T allele interacts with cigarette smoking. Functional studies are required to clarify the potential role of these two SNPs in the pathogenesis of CHD.

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Disclosure statement

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References

[1] Nguyen, T.; Wu, J. J. Relationship between Tumor Necrosis Factor-α Inhibitors and Cardiovascular Disease in Psoriasis: A Review. *Perm. J.* **2014**, *18*, 49–54.

[2] Asifa, G. Z.; Liaquat, A.; Murtaza, I.; Kazmi, S. A. R.; Javed, Q. Tumor Necrosis Factor-Alpha Gene Promoter Region Polymorphism and the Risk of Coronary Heart Disease. *Hindawi* **2013**, *2013*, 1.

[3] Zhang, H.-F.; Xie, S.-L.; Wang, J.-F.; Chen, Y.-X.; Wang, Y.; Huang, T.-C. Tumor Necrosis Factor-Alpha G-308A Gene Polymorphism and Coronary Heart Disease Susceptibility: An Updated Meta-Analysis. *Thrombosis Research* **2011**, *127*, 400–405.

[4] Vendrell, J.; Fernandez-Real, J.-M.; Gutierrez, C.; Zamora, A.; Simon, I.; Bardaji, A.; Ricart, W.; Richart, C. A Polymorphism in the Promoter of the Tumor Necrosis Factor-a Gene (-/308) Is Associated with Coronary Heart Disease in Type 2 Diabetic Patients. *Atherosclerosis* **2003**, *167*, 257–264.

[5] Heidari, M. M.; Khatami, M.; Hadadzadeh, M.; Kazemi, M.; Mahamed, S.; Malekzadeh, P.; Mirjalili, M. Polymorphisms in *NOS3*, *MTHFR*, *APOB* and *TNF-α* Genes and Risk of Coronary Atherosclerotic Lesions in Iranian Patients. *Res. Cardiovasc. Med.* **2016**, *5*, e29134.

[6] Elahi, M. M.; Asotra, K.; Matata, B. M.; Mastana, S. S. Tumor Necrosis Factor Alpha −308 Gene Locus Promoter Polymorphism: An Analysis of Association with Health and Disease. Review. *Biochim. Biophys. Acta* **2009**, *1792*, 163–172.

[7] Srikanth Babu, B. M. V.; Pulla Reddy, B.; Priya, V. H. S.; Munshi, A.; Surekha Rani, H.; Suman Latha, G.; Rao, V. D.; Jyothy, A. Cytokine Gene Polymorphisms in the

Susceptibility to Acute Coronary Syndrome. *Genet. Test. Mol. Biomark.* **2012**, *16*, 359–365.

[8] Garg, P. R.; Saraswathy, K. N.; Kalla, A. K.; Sinha, E.; Ghosh, P. K. Pro-Inflammatory Cytokine Gene Polymorphisms and Threat for Coronary Heart Disease in a North Indian Agrawal Population. *Gene* **2013**, *514*, 69–74.

[9] Zheng, R. L.; Zheng, H.; Jiang, W. L. Tumor Necrosis Factor-Alpha 308G.A Polymorphism and Risk of Rheumatic Heart Disease: A Meta-Analysis. *Sci. Rep.* **2015**, *4*, 4731.

[10] Pravica, V.; Perrey, C.; Stevens, A.; Lee, J.-H.; Hutchinson, I. V. A Single Nucleotide Polymorphism in the First Intron of the Human IFN-g Gene: Absolute Correlation with a Polymorphic CA Microsatellite Marker of High IFN-g Production. *Hum. Immunol.* **2000**, *61*, 863–866.

[11] Dedoussis, G. V.; Panagiotakos, D. B.; Vidra, N. V.; Louizou, E.; Chrysohoou, C.; Germanos, A.; Mantas, Y.; Tokmakidis, S.; Pitsavos, C.; Stefanadis, C.; et al. Association between TNF-α-308G > a Polymorphism and the Development of Acute Coronary Syndromes in Greek Subjects: The CARDIO 2000-GENE Study. *Genet. Med.* **2005**, *7*, 411.

[12] Yang, L.; Fu, C.; Wang, X.; et al. A Meta-Analysis of the Relation between TNF-α G308A Gene Polymorphism and Heart Disease. *Life Science Journal* **2014**, *11*, 204–206.

[13] Cui, G.; Wang, H.; Li, R.; Zhang, L.; Li, Z.; Wang, Y.; Hui, R.; Ding, H.; Wen Wang, D. Polymorphism of Tumor Necrosis Factor Alpha (TNF-Alpha) Gene Promoter, Circulating TNF-Alpha Level, and Cardiovascular Risk Factor for Ischemic Stroke. *J. Neuroinflamm.* **2012**, *9*, 235.

[14] Solhjoo, S.; Mahmoudzadeh-Sagheb, H.; Heidari, Z.; et al. Association between TNF-α (-308 G → a) Gene Polymorphism and Chronic Periodontitis. *ZJRMS* **2014**, *16*, 10–14.

[15] Kim, H.-J.; Kang, S. W.; Chung, J.-H.; Kim, S.-J.; Choe, B.-K. Polymorphisms of the Interferon Gamma Gene and Coronary Artery Disease in the Korean Population. *Mol. Biol. Rep.* **2012**, *39*, 5425–5432.

[16] Bazzaz, J.; Amoli, M. M.; Taheri, Z.; Larijani, B.; Pravica, V.; Hutchinson, I. V. TNF-α and IFN-γ Gene Variation and Genetic Susceptibility to Type 1 Diabetes and Its Microangiopathic Complications. *J. Diabetes Metab. Disord.* **2014**, *13*, 46.

[17] Mann, D. L.; Felker, G. M. *Heart Failure E-Book: A Companion to Braunwald's Heart Disease*: Elsevier Health Sciences: Förlag; 2014.

[18] Al-Kholy, W.; Elsaid, A.; Sleem, A.; Fathy, H.; Elshazli, R.; Settin, A. TNF-α- 308 G > A and IFN-γ +874 A > T gene polymorphisms in Egyptian patients with lupus erythematosus. *Meta Gene* **2016**, *9*, 137–141.

[19] Pravica, V.; Asderakis, A.; Perrey, C.; Hajeer, A.; Sinnott, P. J.; Hutchinson, I. V. In Vitro Production of IFN-gamma Correlates with CA Repeat Polymorphism in the Human IFN-Gamma Gene. *Eur. J. Immunogenet.* **1999**, *26*, 1–3.

[20] Wang, J.; He, Y.; Yang, Y.; Song, T.; Chen, N.; Zhou, Y. Association between the TNF-α G-308A Polymorphism and Risk of Ischemic Heart Disease: A Meta-Analysis. *Int. J. Clin. Exp. Med.* **2015**, *8*, 8880–8892.

[21] Dehkordi, F. R. Association of Inflammatory Mediators with Coronary Artery Disease in Diabetic Patients. *J. Basic Clin. Pathophysiol.* **2014**, *3*, 47–50.

[22] Ebrahimi, M.; Kazemi-Bajestani, S. M. R.; Ghayour-Mobarhan, M.; Ferns, G. A. A. Coronary Artery Disease and Its Risk Factors Status in Iran: A Review. *Iran Red Crescent Med. J.* **2011**, *13*, 610–623.

[23] Sadeghi, R.; Adnani, N.; Erfanifar, A.; Gachkar, L.; Maghsoomi, Z. Premature Coronary Heart Disease and Traditional Risk Factors-Can We Do Better?. *Int. Cardiovasc. Res. J.* **2013**, *7*, 46–50.

[24] Naggar, G.; El-Serogy, H. Is There a Relation between TNF-α 308 Promoter Gene Polymorphism and a Risk of Coronary Artery Disease in Patients with Type 2 Diabetes Mellitus? *Life Sci. J.* **2013**, *10*, 1779–1785.

[25] Chu, H.; Yang, J.; Mi, S.; Bhuyan, S. S.; Li, J.; Zhong, L.; Liu, S.; Tao, Z.; Li, J.; Chen, H.; et al. Tumor Necrosis Factor-Alpha G-308 a Polymorphism and Risk of Coronary Heart Disease and Myocardial Infarction: A Case-Control Study and Meta-Analysis. *JCDR* **2012**, *3*, 84–90.

[26] Esperança, J. C. P.; Miranda, W. R. R.; Netto, J. B.; Lima, F. S.; Baumworcel, L.; Chimelli, L.; Silva, R.; Ürményi, T. P.; Cabello, P. H.; Rondinelli, E.; et al. Polymorphisms in IL-10 and INF-γ Genes Are Associated with Early Atherosclerosis in Coronary but Not in Carotid Arteries: A Study of 122 Autopsy Cases of Young Adults. *BBA Clin*. **2015**, *3*, 214–220.

[27] Sookoian, S.; Garcia, S.; Gianotti, T.; Dieuzeide, G.; Gonzalez, C.; Pirola, C. The G-308A Promoter Variant of the Tumor Necrosis Factor-Alpha Gene Is Associated with Hypertension in Adolescents Harboring the Metabolic Syndrome. *Am. J. Hypertens*. **2005**, *18*, 1271–1275.