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Association between NF-κBI and NF-κBIA polymorphisms and coronary artery disease

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Abstract. Coronary artery disease (CAD) is the leading cause of fatalities worldwide. Nuclear factor (NF)-κB is a transcription factor that controls cell proliferation, differentiation and immunity. To the best of our knowledge, the present study is the first investigation of the association between CAD and *NF-κB1* -94 W/D/*NF-κBIA* 3'-untranslated region (3'-UTR) A→G polymorphisms. The study population comprised 226 CAD patients and 201 controls. There was no significant difference in *NF-κB1A* 3'-UTR A→G in the allele and genotype frequencies between case and control populations. The D allele frequency of *NF-κB1* -94 in the case group was significantly higher compared to the control group (P=0.028, odds ratio=1.37). The genotype frequency of *NF-κB1* -94 DD in the case group was significantly higher compared to the controls (P=0.028). Linkage analysis showed a close linkage among these 2 genes (P<0.001 for case and control), and AD and GD haplotypes were associated with CAD (P<0.001; P=0.015, respectively). *NF-κB1* -94 DD genotype can be a significant risk factor for the development of CAD.

Introduction

Coronary artery diseases (CAD) are multifactorial and they are the leading causes of fatality worldwide (1). Cardiovascular diseases are the causes of 40% of all fatalities in Turkey; by contrast, these diseases are the most common causes of fatality among European men <65 years old and the second most common cause in women (2). Atherosclerosis is the most common form of heart disease, and currently, it is accepted to be a chronic inflammatory disease of the arterial wall. Atherosclerosis is associated with dysregulation of the lipoprotein metabolism, formation of pro-inflammatory lipid peroxidation byproducts and abnormal host immune responses (3).

Nuclear factor (NF)-κB, which is a transcription factor, is used by eukaryotic cells. These cells control cell proliferation, differentiation, immunity and cell survival. NF-κB is therefore involved in numerous proinflammatory processes and in apoptosis. Rela, relb, crel, nfkb1, and nfkb2 genes in mammals encode five NF-κB protein family members, RelA (p65), RelB, c-Rel, p50 and p52, respectively; these form homo- and heterodimeric DNA-binding complexes (4). The human *NF-κB1* gene encodes 2 proteins; p50, with a DNA binding site derived from C-terminal of p105, and the cytoplasmic molecule p105, which has no DNA binding site (5). The p50 homodimer is believed to have the anti-inflammatory effect (6). NF-κBIA (IκBa) encode the inhibitory version of the NF-κB protein; additionally, the *NF-κBIA* gene is similarly regulated by NF-κB (7). Through IκB kinases, external signaling molecules lead to phosphorylation of NF-κBIA on 2 serine sites (IKK). Therefore, following nuclear translocation, active NF-κB binds to promoter regions on DNA and regulates gene transcription in this way (8). To the best of our knowledge, there are no studies concerning the association between CAD and *NF-κBIA* 3'-untranslated region (3'-UTR) A→G; however, there are certain studies in the literature regarding *NF-κBI* -94 W/D polymorphisms. The aim of the present study was to investigate the associations between *NF-κB1* -94 W/D and *NF-κBIA* 3'-UTR A→G polymorphisms and CAD in a Turkish population. Additionally, subgroup and linkage analysis of these genes were also examined for the first time.

Materials and methods

Study population. In the present study, 226 patients with CAD, consisting of 64 females and 162 males, were selected from Cumhuriyet University Hospital (Sivas, Turkey). The study group comprised inhabitants of Sivas, which is in the middle of Turkey and known as the Anatolian region. The control study populations consisted of 201 individuals (74 females and 127 males), based on clinical signs, physical and laboratory examination data and findings of electrocardiography and echocardiography. The control group was selected as they had a negative test result. The diagnosis of CAD was established angiographically in the presence of >50% stenosis in ≥1 of the 3 major coronary arteries or their major branches and all the

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Table I. Demographic and clinical parameters of patients with coronary artery disease and healthy control subjects.

Characteristics	Cases	Controls	OR (95% CI)	P-value
Total, no. (%)	226 (100.00)	201 (100.00)		
Mean age, years ± SD	61.42±6.81	56.89±7.14		0.842
Gender, no. (%)				
Female	64 (28.32)	74 (36.81)		
Male	162 (71.68)	127 (63.19)	1.47 (0.98-2.21)	0.061
Smoking status, no. (%)				
Non-smoker	99 (43.80)	106 (52.73)		
Smoker	127 (56.20)	95 (47.27)	1.43 (0.97-2.09)	0.065
Hypertension, no. (%)				
Absent	84 (37.17)	140 (69.65)		
Present	142 (62.83)	61 (30.35)	3.88 (2.59-5.81)	<0.001
Diabetes				
Absent	146 (64.60)	147 (73.13)		
Present	80 (35.40)	54 (26.87)	1.49 (0.98-2.25)	0.058
Hypercholesterolemia, no. (%)				
Absent	136 (60.18)	137 (70.65)		
Present	90 (39.82)	64 (29.35)	1.41 (0.95-2.11)	0.086

OR, odds ratio; CI, confidence interval; SD, standard deviation.

patients had stable CAD. The study protocol was approved by the Ethics Committee of the Medical School of Cumhuriyet University. Finally, each participant provided written informed consent (no. of Ethics Committee 2011-02/04). The study group consisted of our previous publications; however, there were 226 patient groups in the present study (no. of Ethics Committee 2011-02/04).

Genotyping. Blood samples of 2 ml were collected in blood collection tubes with EDTA. Genomic DNA was extracted from blood leukocytes using the standard phenol-chloroform method. These polymorphisms were genotyped according to a previous study (9). However, 10% of the study population for homozygous wild-type, heterozygous and homozygous mutation of *NF-κB1* -94 ins/delATTG (W/D) and *NF-κBIA* 3'-UTR A→G were confirmed by direct sequencing using an ABI PRISM 377 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis. Statistical analysis was performed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance of the differences in *NF-κB1A* and *NF-κB1* genotypes, and the demographic and clinical parameters of cases and controls were calculated by Pearson's χ^2 test. The t-test was used to evaluate the age distribution between case and control populations. To assess the independent contribution of genotype to CAD, multivariate logistic regression analysis was performed adjusting for age, gender, hypertension, hypercholesterolaemia, smoking habit and diabetes mellitus. For each odds ratio (OR), 95% confidence intervals were calculated. Hardy-Weinberg equilibrium was examined using the Popgene software package (10). Analysis of haplotype frequencies was carried out using the EH programme (11). In all cases, P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical and demographic parameters. The clinical and demographic parameters of patients with CAD and healthy control subjects are presented in Table I. The distribution of age, gender, smoking status, diabetes and hypercholesterolaemia status between the CAD and control groups were not significantly different, except for hypertension (Table I). In addition to the allele and genotype distributions of the CAD and controls, *NF-κB1* and *NF-κBIA* Hardy-Weinberg analysis are presented in Table II.

Allelic frequencies. Distribution of the *NF-κB1* allelic frequency differed significantly between the atherosclerosis cases and controls (P=0.028; OR=1.37). Comparison of the WW genotype with DD genotypes revealed that the variation between CAD patients and controls was statistically significant (P=0.028; OR=2.01). Individuals with *NF-κB1* DD genotype have a 2.73-fold higher risk of atherosclerosis when compared to the case and control group (adjusted OR=2.73) (Table II).

Haplotype analysis. Haplotype analysis was carried out for all the possible haplotypes and all 4 haplotypes, determined by the 2 single-nucleotide polymorphisms, were observed in the study samples. The haplotype frequencies of *NF-κBI* and *NF-κBIA* showed that there was a strong linkage among the 2 genes for the cases and control (for case χ^2=17.64 and P<0.001; for control χ^2=12.89 and P<0.001) (Table II). The distributions of AD and GD haplotype frequencies between

Table II. Risk estimates and frequencies of allele and genotypes for of *NF-κBIA* and *NF-κBI*.

Characteristics	Cases, no. (%)	Controls, no. (%)	P-value	Unadjusted OR (95% Cl)	[a]Adjusted OR (95% Cl)
NF-κBI					
W	266 (58.85)	266 (66.17)		Ref	
D	186 (41.15)	136 (33.83)	0.028	1.37 (1.03-1.81)	
WW	76 (33.63)	85 (42.29)		Ref	0.98 (0.52-1.86)[b]
WD	114 (50.44)	96 (47.76)	0.176	1.33 (0.88-2.00)	
DD	36 (15.93)	20 (9.95)	0.028	2.01 (1.07-3.77)	2.48 (1.19-5.15)[c]
WW+WD	190 (84.07)	181 (90.05)	0.068	0.58 (0.32-1.04)	2.09 (1.25-3.52)[d]
P	0.555	0.361			
χ^2	0.358	0.831			
NF-κBIA					
A	270 (59.73)	264 (65.67)		Ref	
G	182 (40.27)	138 (34.33)	0.074	1.29 (0.98-1.70)	
AA	80 (35.40)	90 (44.78)		Ref	0.95 (0.47-1.88)[e]
AG	110 (48.67)	84 (41.79)	0.066	1.47 (0.97-2.23)	
GG	36 (15.93)	27 (13.43)	0.172	1.50 (0.84-2.69)	1.99 (0.99-3.99)[f]
AA+AG	190 (84.07)	174 (86.57)	0.468	0.82 (0.48-1.40)	7.39 (3.65-14.97)[g]
P	0.885	0.283			
χ^2	0.020	1.150			
Frequencies of haplotypes *NF-κBIA* and *NF-κBI*					
A and W	160 (35.42)	158 (39.13)	Ref	Ref	Ref
A and D	58 (12.80)	20 (5.25)	<0.001	2.86 (1.65-4.98)	3.60 (1.44-8.99)
G and W	110 (24.36)	146 (36.18)	0.092	0.74 (0.53-1.04)	0.67 (0.38-1.19)
G and D	124 (27.42)	78 (19.44)	0.015	1.57 (1.10-2.25)	2.17 (1.17-4.05)

[a]Adjusted for age, gender, hypertension, smoking habit, hypercholesterolemia and diabetes. *NF-κBI*: [b]WW vs. WD+DD (dominant model), [c]WW vs. WD vs. DD (log-additive model), [d]WW+WD vs. DD (recessive model). *NF-κBIA*: [e]AA vs. AG+GG (dominant model), [f]AA vs. AG vs. GG (log-additive model), [g]AA+AG vs. GG (recessive model). *NF-κB*, nuclear factor-κB; OR, odds ratio; CI, confidence interval.

cases and the controls were statistically significant (P<0.001, P=0.015, respectively).

Risk estimates with regards to the parameters. The risk estimates of the *NF-κBI* polymorphisms were calculated in demographic and clinical parameters; as *NF-κBI* was statistically significant (Table III). Male CAD patients had significantly higher frequencies of the DD genotype compared to the controls (P=0.001; OR=4.48). When compared to the controls, CAD patients with hypertension also had significantly higher frequencies of the DD and WD genotypes (P<0.001, OR=4.07; and P=0.023, OR=3.35) (Table III). Hypercholesterolaemia CAD patients had statistically different frequencies of WD and DD genotypes compared to the controls (P=0.024, OR=2.23; and P=0.031, OR=3.11).

Discussion

The association between CAD and *NF-κB1*/*NF-κB1A* polymorphisms was investigated in the present study. While the allele frequency of *NF-κB1A* was 34.33% in the controls, it was reported as 36% in China, 37% in a German population, 45% in a Czech population (12) and 29% in an Australian-Jewish population (13). Arslan and Engin (9) reported this allele frequency as 32.3% in a Turkish population. In the present study, it was determined that there was not a significant association between *NF-κBIA* polymorphisms and CAD (Table II). A different polymorphism of *NF-κBIA* was examined in our previous study and there was a significant association between *NF-κBIA* -826 C/T polymorphisms and CAD (P=0.030) (14). *NF-κBIA* polymorphism has been associated with inflammatory and immune diseases, including Crohn's diseases (CD) (15) and type 2 diabetes. The *NF-κBIA* polymorphism has a weak interaction between NF-κB and IκB; this occurrence had an effect on the expression, structure and function of the protein produced (16).

The frequency of the *NF-κBI* D allele was previously reported to vary from 32 to 54% between certain ethnic populations, compared with 33.83% in the present study (17,18). The frequency of the *NF-κB1* D allele has previously been reported as 33.59% in a Turkish population (9). The present study identified a statistical difference between the *NF-κBI* DD genotype and CAD patients compared with healthy controls in the present study (P=0.028). Individuals with the DD genotype have a 2.73-fold greater risk of CAD compared to those carrying the WW genotype (adjusted OR=2.73)

Table III. Risk estimates of *NF-κBI* polymorphisms in the demographic and clinical parameters.

NF-κBI	Cases, no. (%)	Controls, no. (%)	P-value	OR (95%Cl)
Female				
WW	23 (35.94)	28 (37.83)		
WD	30 (46.87)	32 (43.24)	0.727	1.14 (0.54-2.40)
DD	11 (17.19)	14 (18.91)	0.928	0.95 (0.36-2.50)
Male				
WW	53 (32.72)	57 (44.88)		
WD	84 (51.85)	64 (50.39)	0.172	1.41 (0.86-2.32)
DD	25 (15.43)	6 (4.73)	0.001	4.48 (1.70-11.78)
Smoking				
WW	42 (33.07)	45 (47.37)		
WD	66 (51.97)	40 (42.10)	0.051	1.76 (0.99-3.14)
DD	19 (14.96)	10 (10.53)	0.107	2.03 (0.85-4.87)
Hypertension				
WW	49 (34.51)	41 (67.21)		
WD	73 (51.41)	15 (24.59)	<0.001	4.07 (2.04-8.15)
DD	20 (14.08)	5 (8.20)	0.023	3.35 (1.15-9.70)
Diabetes				
WW	30 (37.50)	26 (48.15)		
WD	33 (41.25)	21 (38.89)	0.424	1.34 (0.61-2.98)
DD	17 (21.25)	7 (12.96)	0.151	1.51 (0.70-6.12)
Hypercholesterolemia				
WW	30 (33.33)	35 (54.69)		
WD	44 (48.89)	23 (35.94)	0.024	2.23 (1.10-4.50)
DD	16 (17.78)	6 (9.37)	0.031	3.11 (1.08-8.95)

NF-κBI, nuclear factor-κBI; OR, odds ratio; CI, confidence interval.

(Table II). However, there was no significant difference in the *NF-κB1* WD genotype frequencies between CAD patient and control populations compared to those carrying the WW genotype (P=0.176) (Table II). Liang *et al* (19) conducted a meta-analysis of different ethnic groups with inflammatory bowel disease, which includes ulcerative colitis (UC) and CD. The study reported a significant genetic association of the *NF-κB1* gene polymorphism with UC, but not CD. Another meta-analysis, composed of different ethnic groups, reported that a significant association was identified between the *NF-κB1* polymorphism and autoimmune and inflammatory diseases in the Asian population (20). Karban *et al* (21) stated that, when compared with the W allele *in vitro*, the *NF-κB1* gene with the D allele exhibited reduced transcription activity. Vogel *et al* (22) identified that the p50 depletion of the del-allele affects the anti-inflammatory response. The study identified that patients with the del-allele have a higher risk of CAD. The haplotype analysis was also examined in Table II. By contrast, a significant association was determined of the AD and GD haplotypes between the case and control groups (P<0.001 adjusted OR=3.60; and P=0.015, adjusted OR=2.17, respectively). Individuals with the *NF-κB1* D allele may not produce an adequate immune response against inflammation due to the low transcriptional activity of the *NF-κB* gene.

Subgroups of the *NF-κB1* polymorphisms were studied as this polymorphism is significant for CAD (Table III). There was a statistically significant difference between case and control populations in males, hypercholesterolaemia and hypertension (P=0.001, P=0.031 and P=0.023, respectively) (Table III). Male individuals have a 4-fold higher risk of CAD compared to female individuals (for males, OR=4.48; for females, OR=0.95). The difference between gender results from certain risks and hormonal factors during development periods (23). There was a statistically significant difference between case and control in the hypercholesterolaemia comparison of the WW genotype, WD and DD genotypes (P=0.031 and P=0.024, respectively). Low-grade inflammation is mainly coordinated by NF-κB; it is also known to be associated with an altered lipid profile (24). In prospective studies, it was reported that plasma C-reactive protein (CRP) levels are risk factors for CAD, and CRP polymorphisms were associated with high CRP levels (25). Cha-Molstad *et al* (26) reported that the p50 dimer of NF-κB activates transcription of CRP. Vogel *et al* (22) identified that the del-allele carriers had lower CRP levels. There was a statistically significant difference between case and controls for hypertension in the comparison of the WW genotype and WD and DD genotypes (P<0.001 and P=0.023). Individuals with the DD genotype have a 3-fold higher risk of CAD compared to WW (OR=3.35) (Table III).

Hypertension, diabetes and hypercholesterolaemia are intermediate variables between inflammation and CAD (22).

In conclusion, the associations between CAD and *NF-κBI* -94 W/D and *NF-κBIA* 3'-UTR A→G polymorphisms were investigated for the first time in a Turkish population. The present study indicated that CAD is associated with the *NF-κBI* -94 W/D but not with *NF-κBIA* 3'-UTR A→G. The *NF-κBI* DD genotype may be a significant risk factor for developing CAD. As mentioned previously, linkage analysis was also performed, and the results of this analysis showed that there was a strong linkage between these 2 genes, and the AD and GD haplotypes were associated with CAD. Subgroup analyses of *NF-κBI* -94 W/D identified that there was a statistically significant difference between case and control in males, hypertension and hypercholesterolaemia.

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