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The TNF-α G-308A polymorphism is associated with C-reactive protein levels: The HERITAGE Family Study

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Abstract

Objective: Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), stimulate the release of C-reactive protein (CRP). We investigated the association between the TNF-α G-308A polymorphism and plasma CRP levels.
Methods: Subjects were 456 White (225 men, 231 women) and 232 Black (83 men, 149 women) healthy adults who underwent a 20-week standardized exercise program in the HERITAGE Family Study. The TNF-α gene promoter polymorphism was determined using PCR amplification followed by *NcoI* digestion. Plasma CRP was measured using a high-sensitivity assay.
Results: Genotype frequencies were in Hardy Weinberg equilibrium. After adjustment for age, smoking, alcohol consumption, maximal oxygen uptake and, in women, hormone use, the AA homozygotes for the G-308A polymorphism had higher baseline CRP levels than other genotypes in White and Black men ($P<0.001$ and $P=0.044$, respectively) and in Black women ($P=0.032$). Body mass index partly explained these associations in Blacks. The exercise program results provided further evidence for an association with the polymorphism. Among those with high CRP at baseline (≥3.0 mg/L), regular exercise decreased CRP less in AA homozygotes than in other genotypes ($P=0.043$).
Conclusion: The AA genotype of the TNF-α G-308A polymorphism is associated with higher plasma CRP levels and less favorable CRP response to regular exercise.

Keywords: Tumor necrosis factor alpha; Polymorphism; C-reactive protein; Inflammation; Adiposity

1. Introduction

Circulating C-reactive protein (CRP) concentrations reflect the inflammatory status of an individual (Libby et al., 2002; Pearson et al., 2003). Systemic or local inflammation triggers the production of pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α). These cytokines stimulate the production of IL-6, which induces hepatic production of acute-phase reactants including CRP (Libby et al., 2002). Elevated serum levels of CRP increase the risk of acute myocardial infarction, ischemic stroke, peripheral artery disease, type 2 diabetes, and metabolic syndrome (Hu et al., 2004; Koenig et al., 1999; Ridker et al., 2003; Rost et al., 2001). Moreover, serum TNF-α levels have been shown to be associated with carotid atherosclerosis in healthy men (Skoog et al., 2002), and an increased risk of recurrent coronary events in survivors of myocardial infarction (Ridker et al., 2000).

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Elevated TNF-α levels are associated with many inflammatory diseases, such as rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD) (Baugh and Bucala, 2001; Furst et al., 2003), and TNF-α inhibitors are widely used to treat them (Baugh and Bucala, 2001; Furst et al., 2003).

Several polymorphisms have been identified in the gene encoding TNF-α. Among them, a guanine (G) to adenine (A) substitution located at position − 308 in the promoter region affects the rate of transcription of the TNF-α gene, the A allele being associated with higher levels of transcription (Kroeger et al., 1997; Wilson et al., 1997). Some, but not all, studies have indicated an important role for the G-308A variant in the pathogenesis of obesity and obesity-assoeiated insulin resistance (Brand et al., 2001; Fernandez-Real et al., 1997; Hoffstedt et al., 2000; Walston et al., 1999). In a recent study in patients with RA, those with the TNF-α-308 GG genotype were better TNF-α inhibitor infliximab responders than were patients with the AA or AG genotype (Mugnier et al., 2003).

Polymorphisms in the TNF-α gene could affect downstream signaling of TNF-α and thus circulating CRP levels. There are no previous studies on the TNF-α G-308A polymorphism and plasma CRP levels in healthy subjects. We investigated the relationship before and after an exercise program in White and Black men and women who participated in the HERITAGE Family Study.

2. Methods

2.1. Study design and subjects

The HERITAGE Family Study is a multicenter study, carried out by a consortium of five universities in the United States and Canada (Bouchard et al., 1995). The study design, sampling, as well as inelusion and exclusion criteria have been described previously (Bouchard et al., 1995). In brief, the offspring were required to be aged ≥ 17 years and the parents ≤ 65 years. The subjects were required to be sedentary, defined as not having engaged in regular physical activity over the previous six months, and free of diabetes, eardiovascular diseases or other chronic diseases that would prevent their participation in an exercise-training program. The exclusion criteria included severe obesity (BMI>40 kg/m^2), unless the subject could meet the demands of the exercise program, hypertension (resting blood pressure>159/99 mmHg) or the use of medication for hyperglycemia, hyperlipidemia or hypertension. Thus, the subjects did not have any diseases or conditions that could have affected the inflammatory process. The study protocol was approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant. The present analyses included 456 Whites (225 men and 231 women) and 232 Blacks (83 men and 149 women) for whom complete data were available on the TNF-α G-308A polymorphism and plasma CRP levels. One White female and one Black woman were excluded due to high CRP values (56.7 and 88.0 mg/L, respectively) suggesting the presence of an acute infection.

2.2. Exercise training

The 20-week exercise-training program has been described in detail previously (Bouchard et al., 1995). Briefly, the subjects exercised 3 times per week on cycle ergometers in the laboratory. Exercise intensity was customized for each subject based on the relationship between heart rate and oxygen uptake measured at baseline. During the first 2 weeks, the subjects exercised for 30 min per session at a heart rate corresponding to 55% of the maximal oxygen uptake (VO_{2max}) measured at baseline. Duration was gradually increased to 50 min per session and heart rate was increased to the level corresponding to 75% of the baseline VO_{2max}. This level was sustained for the last 6 weeks. Heart rate was monitored during all exercise sessions by a computerized cycle ergometer system, which adjusted ergometer resistance to maintain the target heart rate. The subjects were instructed not to change their lifestyle during the exercise intervention.

2.3. Measurement of CRP

Plasma CRP at baseline and after the 20-week exereise-training program was measured with a high-sensitivity solid-phase chemilumineseent immunometric assay (IMMULITE 2000 High Sensitivity CRP, Diagnostic Products Corporation, Los Angeles, CA) implemented on an automated immunoassay instrument (Diagnostie Products Corporation, Los Angeles, CA). In a sample of 48 blind duplieates, the intra-class correlation was 0.98 and the coeffieient of variation was 6.4%.

2.4. Other measurements

Anthropometric and body density measurements have been described in detail previously (Wilmore et al., 1997). Body mass index (BMI) was computed as the ratio of body weight divided by body height squared (kg/m^2). Total body fat mass was determined from body density measurements using hydrostatic weighing (Wilmore et al., 1997) according to Behnke and Wilmore (1974). Smoking status and alcohol consumption were assessed by a health habit questionnaire (Bouchard et al., 1995). The use of hormone replacement therapy or oral contraceptives was assessed with a questionnaire (Bouchard et al., 1995). Maximal oxygen uptake was determined during a maximal exercise stress test on a cycle ergometer (Ergo-Metrics 800S from Sensor Medics) (Bouchard et al., 1995).

2.5. Genotyping

The TNF-α G-308A polymorphism was detected after PCR amplification by a *NcoI* enzyme digestion (Wilson et al., 1992). Genomic DNA was prepared from lymphoblastoid cell lines. 250 ng of genomic DNA was amplified using 1.2 μM concentrations of primers F and R in a total volume of 30 μL containing 0.5 U *Taq* DNA polymerase (Pharmacia Biotech), 200 μM of dNTP, 2 μL of 10× reaction buffer (500 mM KCL, 15 mM $MgCl_2$, and 100 mM Tris HCl). Cycling: 94 °C for 3 min, 60 °C for 1 min and 72 °C for 1 min, followed by 38 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, with a final cycle of 94 °C for

Table 1
Baseline characteristics of the subjects

	Whites (n=456)		Blacks (n=232)	
	Men (n=225)	Women (n=231)	Men (n=83)	Women (n=149)
Plasma high sensitivity C-reactive protein (mg/L)	1.8 (2.4)	3.0 (3.9)	2.0 (2.1)	3.8 (4.6)
Age (years)	36.9 (15.0)	35.8 (14.2)	34.9 (12.4)	33.8 (11.4)
Body mass index (kg/m^2)	26.8 (4.9)	25.2 (5.0)	27.5 (5.2)	28.6 (6.2)
Body fat mass (kg)[a]	20.3 (10.7)	21.4 (11.1)	21.1 (10.6)	28.6 (12.0)
VO_{2max} (mL/min)	3023.3 (591.4)	1901.8 (352.8)	2736.8 (511.6)	1744.3 (353.0)
Cigarette smoking (%)				
Current	13.4	16.5	4.9	12.2
Former	27.2	20.8	26.8	14.3
Never	59.4	62.8	68.3	73.5
Alcohol consumption (g/day)	8.5 (13.7)	4.0 (6.2)	4.2 (6.9)	1.7 (5.1)
Hormone use (%)[b]	–	39.8	–	22.2

Data are presented as mean (standard deviation) or %.
[a] By underwater weighing.
[b] Hormone use, hormone replacement therapy or use of oral contraceptives.

1 min, 60 °C for 1 min and 72 °C for 5 min. The primers for TNF-α amplification were as follows: F: 5 -AGGCAATAGGTTTTGAGGGCCAT-3 ; R: 5 -TCCTTGGTGGAGAAACCCATAAG-3 . The amplified product was digested with 10 U of *NcoI* at 37 °C in water bath. The digested products were separated by electrophoresis on a 9% polyacrylamide gel. The bands were visualized with ethidium bromide.

2.6. Statistical analyses

All analyses were performed using the SAS statistical analysis package (version 8.2, SAS Institute, Cary, NC, USA). Data are presented as mean (standard deviation) for continuous variables and number of subjects (%) for categorical variables. Because of its skewed distribution, plasma CRP was log-transformed. A chi-squared test was used to assess whether the observed genotype frequencies were in Hardy Weinberg equilibrium. The stratification of CRP levels as <3.0 mg/L (low) and ≥3.0 mg/L (high) is based on a recent AHA/CDC Scientific Statement (Pearson et al., 2003). The Fisher exact test was used to assess the frequencies of the genotypes among subjects with low and high CRP. Associations between the polymorphism and CRP levels were analysed using the MIXED procedure of SAS. Non-independence among family members was adjusted for by using a sandwich estimator which asymptotically yields the same parameter estimates as ordinary least squares or regression

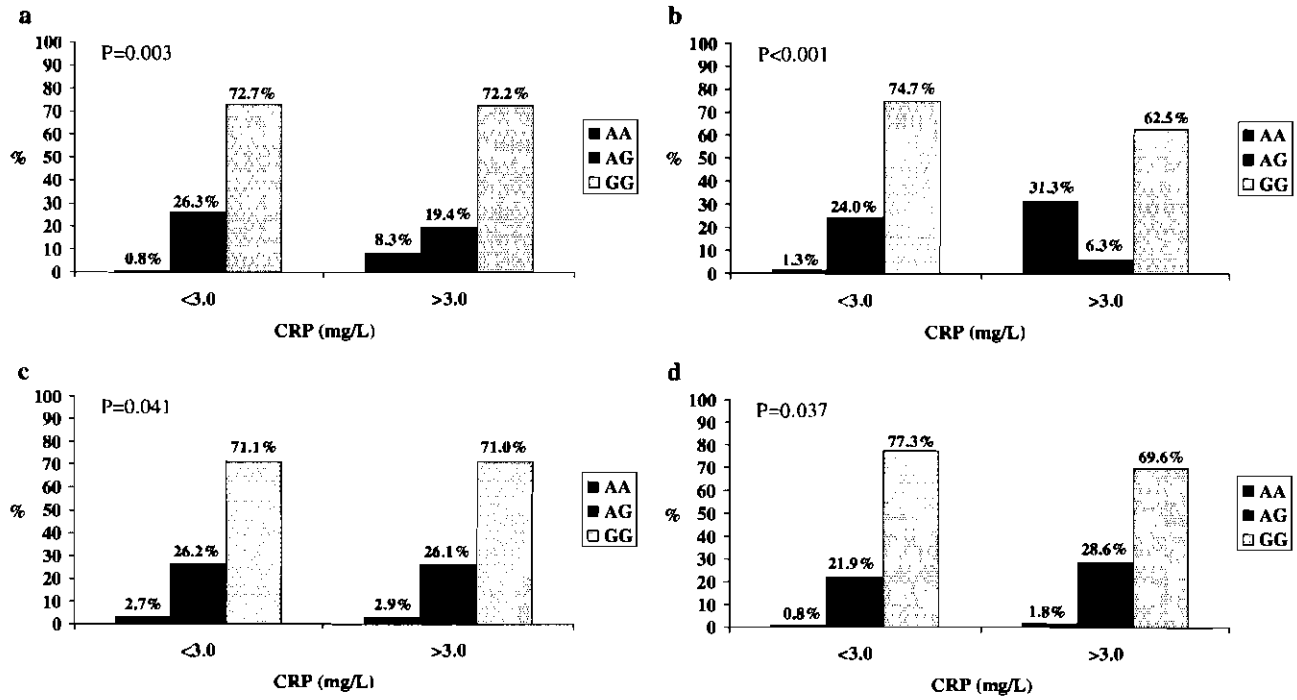

Fig. 1. a–d. TNF-α genotype distributions by baseline CRP level in White men (a), Black men (b), White women (c), and Black women (d).

Table 2
The associations between the TNF-α G-308A polymorphism and CRP levels at baseline and after a 20-week exercise training program

	Baseline		Post-training	
	Model 1	Model 2	Model 1	Model 2
	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)
White men (n=225)				
AA (n=5)	4.31 (0.59)	3.77 (0.34)	4.19 (0.54)	3.87 (1.71)
AG (n=57)	1.87 (0.43)	1.68 (0.39)	2.09 (0.39)	1.97 (0.40)
GG (n=163)	2.22 (0.46)	2.03 (0.43)	2.34 (0.37)	2.19 (0.35)
P for difference between groups	<0.001	0.001	<0.001	0.018
White women (n=231)				
AA (n=6)	1.01 (1.44)	1.49 (1.08)	3.81 (1.90)	4.26 (2.10)
AG (n=57)	3.81 (0.86)	4.23 (0.82)	3.24 (0.67)	3.69 (0.61)
GG (n=168)	3.13 (0.57)	3.20 (0.52)	3.05 (0.44)	3.08 (0.39)
P for difference between groups	0.263	0.073	0.788	0.811
Black men (n=83)				
AA (n=6)	3.46 (0.83)	3.24 (0.81)	4.75 (1.20)	4.74 (1.22)
AG (n=17)	1.73 (0.51)	1.93 (0.50)	2.20 (0.76)	2.21 (0.77)
GG (n=60)	2.64 (0.37)	2.64 (0.36)	2.51 (0.54)	2.51 (0.54)
P for difference between groups	0.044	0.138	0.121	0.186
Black women (n=149)				
AA (n=2)	4.13 (1.60)	3.23 (1.51)	5.56 (0.99)	4.30 (1.01)
AG (n=36)	2.04 (0.79)	3.42 (0.74)	3.07 (0.71)	3.66 (0.64)
GG (n=111)	2.38 (0.50)	3.92 (0.55)	4.02 (0.53)	4.35 (0.51)
P for difference between groups	0.032	0.709	0.010	0.249

Data are presented as mean (standard error of the mean).
Model 1: Adjusted for age, smoking, alcohol consumption, maximal oxygen consumption, and, in women, hormone use.
Model 2: Adjusted additionally for BMI.

methods, but the standard errors and consequently hypothesis tests are adjusted for the dependencies. The method is general, assuming the same degree of dependency among all members within a family. For Black men, P-values could not be obtained in some analyses when using the sandwich estimator in the model due to low frequencies in some cells of the analysis. In these cases, the analysis was performed using MIXED procedure without the sandwich estimator. P-values of <0.05 were considered statistically significant.

3. Results

Genotype frequencies for AA, AG and GG were 2.4% (n=11), 25.0% (n=114) and 72.6% (n=331) in Whites and 3.5% (n=8), 22.8% (n=53) and 73.7% (n=171) in Blacks, respectively, and they were in Hardy–Weinberg equilibrium. Baseline characteristics of the subjects are shown in Table 1. Blacks had higher CRP levels compared to Whites and women compared to men. The percentage of normal weight (BMI<25 kg/m^2), overweight (25–30 kg/m^2), and obese (≥30 kg/m^2) subjects was 41.6%, 34.7%, and 23.7%, respectively, in White men. The corresponding percentages were 57.4%, 27.3%, and 15.2% in White women, 36.3%, 38.5%, and 25.3% in Black men, and 37.0%, 28.3%, and 34.8% in Black women. The percentage of current smokers was 13.4%, 16.5%, 4.9%, and 12.2% in White men and women and in Black men and women, respectively. 39.8% of White and 22.2% of Black women used hormone replacement therapy or oral contraceptives.

The genotype frequencies differed between those who had plasma CRP of <3.0 mg/L and ≥3.0 mg/L (Fig. 1). In White men, 8.3% of those who had high CRP were homozygous for the A allele, but only 0.8% of those with low CRP level had the AA genotype (P=0.003). In Black men, 31.3% of those who had high CRP were AA homozygotes compared to 1.3% of those with low CRP level (P<0.001). In women, the difference was smaller (Fig. 1).

The associations between the TNF-α G-308A polymorphism and CRP levels at baseline are presented in Table 2. After adjustment for the covariates (age, smoking, alcohol consumption, maximal oxygen consumption and, in women, hormone use), the G-308A polymorphism was associated with CRP level in White men (P<0.001), Black men (P=0.044) and Black women (P=0.032) (Table 2). Further adjustment for BMI did not affect the association in white men (P=0.001), but weakened it in Blacks (Table 2). When adjusting for fat mass instead of BMI, the results were similar (data not shown).

To further explore the lack of association in White women, white mothers and daughters were analyzed separately. The mean CRP levels in mothers were 7.69 (SEM, 2.63), 4.50 (0.76), and 3.18 (0.48) (P=0.001) in AA, AG, and GG groups, respectively. The corresponding values in daughters were 0.62 (1.95), 3.54 (0.69), and 2.52 (0.38) (P=0.001). The mothers had higher BMI (mean BMI [SD], 27.6 [4.8] kg/m^2), and had more fat mass (27.2 [10.6] kg) compared to the daughters (BMI 23.8 [4.5] kg/m^2, fat mass 18.2 [10.2] kg). Consequently, the association in mothers was largely explained by fatness. The mean values became 7.95 (0.97), 7.60 (0.94), and 6.64 (0.62) (P=0.182) in AA, AG, and GG after further adjustment for fat mass. In daughters, the finding that the AA genotype was associated with lower baseline CRP levels in daughters remained after further adjustment for fat mass with mean values of 0.39 (0.61), 3.31 (0.68), and 2.27 (0.52) (P=0.001) in AA, AG, and GG, respectively.

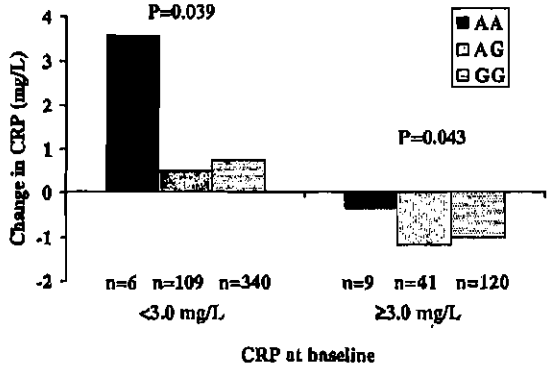

Fig. 2. Exercise-training induced changes in plasma CRP in individuals with low (<3.0 mg/L), and high (≥3.0 mg/L) baseline CRP adjusted for age, sex, race, smoking status, alcohol consumption, maximal oxygen uptake, hormone use, and body fat mass.

The associations between the TNF-α G-308A polymorphism and CRP levels after the 20-week exercise training intervention program are also shown in Table 2. After adjustment for the covariates, G-308A polymorphism was associated with CRP level in White men and Black women ($P<0.001$ and $P=0.010$, respectively). In Black men, the difference between the genotypes was not statistically significant ($P=0.121$). However, Black men who were homozygous for the A allele had markedly higher CRP levels compared to G allele carriers. After further adjustment for BMI, the association remained statistically significant in White men ($P=0.018$) (Table 2).

We have earlier found in the same subjects that regular exercise reduces CRP only among those who have high baseline CRP (≥3.0 mg/L) levels (Lakka et al., 2005). Therefore, we analyzed whether the TNF-α gene G-308A polymorphism contributes to variability in regular exercise-induced changes in CRP levels as a function of baseline CRP level (<3.0 mg/L and ≥3.0 mg/L). After adjustment for age, sex, race, smoking, alcohol consumption, maximal oxygen consumption, hormone use, and body fat mass, CRP decreased in those who had high CRP at baseline (Fig. 2). However, CRP decreased more in G allele carriers than in AA homozygotes ($P=0.043$ for difference between genotypes). Among those with low baseline values, CRP increased slightly in G allele carriers, but it increased markedly in AA homozygotes ($P=0.039$) (Fig. 2).

4. Discussion

In healthy previously sedentary young and middle-aged adults, the AA genotype of the TNF-α G-308A polymorphism was associated with increased plasma CRP levels in White and Black men and in Black women, suggesting that the polymorphism contributes to variability in plasma CRP levels. The analyses after a 20-week exercise training program provided further evidence for our finding. The CRP-decreasing effect of exercise training was evident in those who had high baseline CRP levels, e.g. greater than or equal to 3.0 mg/L, in all genotype groups. However, among those with low CRP at baseline, CRP increased markedly in AA homozygotes. The AA genotype for the G-308A polymorphism was associated with increased plasma CRP levels in White and Black men and in Black women both in the sedentary state and after a 20-week exercise program even after adjustment for age, smoking, alcohol consumption, maximal oxygen consumption, and, in women, hormone use. In our study, individuals who had diseases or took medications that could affect inflammation and thus confound the findings were excluded.

To the best of our knowledge, there are no previous studies on the relationship between the TNF-α gene G-308A polymorphism and plasma CRP levels in healthy subjects. This polymorphism has been associated with obesity and obesity-associated insulin resistance, but findings have not been consistent (Brand et al., 2001; Fernandez-Real et al., 1997; Hoffstedt et al., 2000; Walston et al., 1999). Adiposity is clearly a strong determinant of chronic inflammation measured by high-sensitivity CRP (Lemieux et al., 2001). Body mass index and fat mass partly explained the association with the gene marker in Blacks. Interestingly, however, adiposity did not account for the association in Whites. Further studies are needed to define differences in the association between the TNF-α G-308A polymorphism and CRP among races.

A few large cross-sectional epidemiological studies have found an inverse association between self-reported physical activity and markers of systemic inflammation such as serum CRP (Abramson and Vaccarino, 2002; Albert et al., 2004; Ford, 2002; Geffken et al., 2001; Pitsavos et al., 2003; Wannamethee et al., 2002). Small clinical trials have suggested that exercise training suppresses inflammation in people who have high CRP at baseline (Smith et al., 1999; You et al., 2004), but some participants had conditions or medications that may have affected inflammation and consequently the findings. In the present study, in those with high CRP at baseline, after adjustment for confounders including fat mass, CRP decreased more in G allele carriers than in AA homozygotes. Among those with low CRP at baseline, CRP increased slightly in G allele carriers but markedly in AA homozygotes. Thus, the G-308A polymorphism contributed also to variability in plasma CRP response to exercise training.

The TNF-α G-308A polymorphism has been associated with RA (Mugnier et al., 2003), IBD (Gonzalez et al., 2003; Vatay et al., 2003), inflammatory lung diseases including childhood asthma (Winchester et al., 2000), chronic obstructive pulmonary disease (Sakao et al., 2001), sarcoidosis (Seitzer et al., 1997), coal miners pneumoconiosis (Zhai et al., 1998), and alveolitis in farmer s lung patients (Schaaf et al., 2001), as well as in contact dermatitis (Allen et al., 2000) and prostate cancer (Oh et al., 2000). The G-308A polymorphism of the TNF-α gene has been studied with regard to inflammatory diseases and anti-TNF-α treatment strategies. In a recent study in patients with RA, those with a GG genotype were better responders to TNF-α inhibitor infliximab than were patients with the AA or AG genotype, suggesting that TNF-α G-308A genotyping may be a useful tool for predicting response to infliximab treatment (Mugnier et al., 2003). TNF-α inhibitors are expensive, need to be administered parenterally, with subsets of patients who do not respond to the currently available TNF-α blocking agents. Genotyping of the G-308A polymorphism of the TNF-α gene may be a useful tool to predict inflammatory diseases and to select patients for anti-TNF-α treatment.

The strengths of the current investigation include the large study sample of White and Black men and women, the standardized exercise program, the exclusion of individuals who had diseases or medications that could affect inflammation and thus confound the analyses, and the comprehensive assessment of other confounding factors.

The small number of AA homozygotes is a limitation, although the genotype and allele frequencies were similar to those reported in previous studies of this polymorphism. As the AA genotype is relatively rare, A allele carriers have been compared to GG homozygotes in most studies. However, the phenotypes of individuals with AG or GG genotype have appeared quite similar in many previous reports, and AA homozygotes seem to differ more clearly from G allele carriers. This was also the case in the present study. In our study, there

were altogether 20 AA homozygotes. Despite the small number of subjeets in the AA groups, we found that AA homozygotes had eonsistently higher CRP levels before and after the exereise intervention program, exeept in White women at baseline. AA homozygotes also had a less favorable exercise-indueed ehange eompared to G allele earriers. In White women, we found that the mothers who were homozygous for the A allele had higher baseline CRP levels eompared to daughters. The adverse effects of mild alterations in TNF-α expression may beeome apparent in older age (Heijmans et al., 2002). Age differenees have also been suggested to aceount for the ineonsistent results in studies on the association between the G-308A polymorphism and insulin sensitivity (Fernandez-Real et al., 1997; Heijmans et al., 2002). Unfortunately, the small number of AA homozygotes did not allow us to perform more detailed subgroup analyses.

5. Conclusions

In summary, the present study shows indicates or suggests several findings:

The AA genotype of the G-308A polymorphism of the TNF-α gene is associated with increased plasma CRP levels in healthy, previously sedentary young and middle-aged Whitc and Blaek men and in Black women both before and after an exercise training program.

This polymorphism appears to eontribute to the variability in plasma CRP levels.

The AA homozygotes and G allele carriers have different effects with regard to the response of plasma CRP level to an exercise program.

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References

Abramson, J.L., Vaccarino, V., 2002. Relationship between physical aetivity and inflammation among apparently healthy middle-aged and older US adults. Arch. Intern. Med. 162, 1286 1292.

Albert, M.A., Glynn, R.J., Ridker, P.M., 2004. Effect of physical activity on serum C-reactive protein. Am. J. Cardiol. 93, 221 225.

Allen, M.H., Wakelin, S.H., Holloway, D., Lisby, S., Baadsgaard, O., Barker, J.N., McFadden, J.P., 2000. Association of TNFA gene polymorphism at position-308 with susceptibility to irritant contact dermatitis. Immunogenetics 51, 201 205.

Baugh, J.A., Bucala, R., 2001. Mechanisms for modulating TNF alpha in immune and inflammatory disease. Curr. Opin. Drug Discov. Dev. 4, 635 650.

Behnke, A.R., Wilmore, J.H., 1974. Evaluation and Regulation of Body Build and Composition. Prentice-Hall, Englewood Cliffs, NJ.

Bouchard, C., Leon, A.S., Rao, D.C., Skinner, J.S., Wilmore, J.H., Gagnon, J., 1995. The HERITAGE family study. Aims, design, and measurement protocol. Med. Sci. Sports Exerc. 27, 721 729.

Brand, E., Schorr, U., Kunz, I., Kertunen, E., Ringel, J., Distler, A., Sharma, A.M., 2001. Tumor necrosis factor-alpha-308 G/A polymorphism in obese Caucasians. Int. J. Obes. Relat. Metab. Disord. 25, 581 585.

Fernandez-Real, J.M., Gutierrez, C., Ricart, W., Casamitjana, R., Fernandez-Castaner, M., Vendrell, J., Richart, C., Soler, J., 1997. The TNF-alpha gene Nco I polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. Diabetes 46, 1468 1472.

Ford, E.S., 2002. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 13, 561 568.

Furst, D.E., Breedveld, F.C., Kalden, J.R., Smolen, J.S., Burmester, G.R., Dougados, M., Emery, P., Gibofsky, A., Kavanaugh, A.F., Keystone, E.C., et al., 2003. Updated consensus statement on biological agents for the treatment of rheumatoid arthritis and other immune mediated inflammatory diseases. Ann. Rheum. Dis. 62 (Suppl 2), ii2 ii9.

Geffken, D.F., Cushman, M., Burke, G.L., Polak, J.F., Sakkinen, P.A., Tracy, R.P., 2001. Association between physical activity and markers of inflammation in a healthy elderly population. Am. J. Epidemiol. 153, 242 250.

Gonzalez, S., Rodrigo, L., Martinez-Borra, J., Lopez-Vazquez, A., Fuentes, D., Nino, P., Cadahia, V., Saro, C., Dieguez, M.A., Lopez-Larrea, C., 2003. TNF-alpha-308A promoter polymorphism is associated with enhanced TNF-alpha production and inflammatory activity in Crohn's patients with fistulizing disease. Am. J. Gastroenterol. 98, 1101 1106.

Heijmans, B.T., Westendorp, R.G., Droog, S., Kluft, C., Knook, D.L., Slagboom, P.E., 2002. Association of the tumour necrosis factor alpha-308G/A polymorphism with the risk of diahetes in an elderly population-based cohort. Genes Immun. 3, 225 228.

Hoffstedt, J., Eriksson, P., Hellstrom, L., Rossner, S., Ryden, M., Arner, P., 2000. Excessive fat accumulation is associated with the TNF alpha-308 G/A promoter polymorphism in women but not in men. Diabetologia 43, 117 120.

Hu, F.B., Meigs, J.B., Li, T.Y., Rifai, N., Manson, J.E., 2004. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 53, 693 700.

Koenig, W., Sund, M., Frohlich, M., Fischer, H.G., Lowel, H., Doring, A., Hutchinson, W.L., Pepys, M.B., 1999. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 99, 237 242.

Kroeger, K.M., Carville, K.S., Abraham, L.J., 1997. The − 308 tumor necrosis factor alpha promoter polymorphism effects transcription. Mol. Immunol. 34, 391 399.

Lakka, T.A., Lakka, H.M., Rankinen, T., Leon, A.S., Rao, D.C., Skinner, J.S., Wilmore, J.H., Bouchard, C., 2005. Effect of exercise training on plasma levels of C-reactive protein in healthy adults: the HERITAGE Family Study. Eur. Heart J. (Jun 29 Epub ahead of print).

Lemieux, I., Pascot, A., Prud'homme, D., Almeras, N., Bogaty, P., Nadeau, A., Bergeron, J., Despres, J.P., 2001. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler. Thromb. Vasc. Biol. 21, 961 967.

Libby, P., Ridker, P.M., Maseri, A., 2002. Inflammation and atherosclerosis. Circulation 105, 1135 1143.

Mugnier, B., Balandraud, N., Darque, A., Roudier, C., Roudier, J., Reviron, D., 2003. Polymorphism at position − 308 of the tumor necrosis factor alpha gene influences outcome of infliximab therapy in rheumatoid arthritis. Arthritis Rheum. 48, 1849 1852.

Oh, B.R., Sasaki, M., Perinchery, G., Ryu, S.B., Park, Y.I., Carroll, P., Dahiya, R., 2000. Frequent genotype changes at −308, and 488 regions of the tumor necrosis factor-alpha (TNF-alpha) gene in patients with prostate cancer. J. Urol. 163, 1584 1587.

Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon 3rd, R.O., Criqui, M., Fadl, Y.Y., Fortmann, S.P., Hong, Y., Myers, G.L., et al., 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107, 499 511.

Pitsavos, C., Chrysohoou, C., Panagiotakos, D.B., Skoumas, J., Zeimbekis, A., Kokkinos, P., Stefanadis, C., Toutouzas, P.K., 2003. Association of leisure-

time physical activity on inflammation markers (C-reactive protein, white cell blood count, serum amyloid A, and fibrinogen) in healthy subjects (from the ATTICA study). Am. J. Cardiol. 91, 368 370.

Ridker, P.M., Rifai, N., Pfeffer, M., Saeks, F., Lepage, S., Braunwald, E., 2000. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation 101, 2149 2153.

Ridker, P.M., Buring, J.E., Cook, N.R., Rifai, N., 2003. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy American women. Circulation 107, 391 397.

Rost, N.S., Wolf, P.A., Kase, C.S., Kelly-Hayes, M., Silbershatz, H., Massaro, J.M., D'Agostino, R.B., Franzblau, C., Wilson, P.W., 2001. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. Stroke 32, 2575 2579.

Sakao, S., Tatsumi, K., Igari, H., Shino, Y., Shirasawa, H., Kuriyama, T., 2001. Association of tumor necrosis factor alpha gene promoter polymorphism with the presence of chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 163, 420 422.

Schaaf, B.M., Seitzer, U., Pravica, V., Aries, S.P., Zabel, P., 2001. Tumor necrosis factor-alpha-308 promoter gene polymorphism and increased tumor necrosis factor serum bioactivity in farmer's lung patients. Am. J. Respir. Crit. Care Med. 163, 379 382.

Seitzer, U., Swider, C., Stuber, F., Suchnicki, K., Lange, A., Richter, E., Zabel, P., Muller-Quernheim, J., Flad, H.D., Gerdes, J., 1997. Tumour necrosis factor alpha promoter gene polymorphism in sarcoidosis. Cytokine 9, 787 790.

Skoog, T., Dichtl, W., Boquist, S., Skoglund-Andersson, C., Karpe, F., Tang, R., Bond, M.G., de Faire, U., Nilsson, J., Eriksson, P., Hamsten, A., 2002. Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. Eur. Heart J. 23, 376 383.

Smith, J.K., Dykes, R., Douglas, J.E., Krishnaswamy, G., Berk, S., 1999. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. JAMA 281, 1722 1727.

Vatay, A., Bene, L., Kovaes, A., Prohaszka, Z., Szalai, C., Romics, L., Fekete, B., Karadi, I., Fust, G., 2003. Relationship between the tumor necrosis factor alpha polymorphism and the serum C-reactive protein levels in inflammatory bowel disease. Immunogenetics 55, 247 252.

Walston, J., Seibert, M., Yen, C.J., Cheskin, L.J., Andersen, R.E., 1999. Tumor necrosis factor-alpha-238 and -308 polymorphisms do not associated with traits related to obesity and insulin resistance. Diabetes 48, 2096 2098.

Wannamethee, S.G., Lowe, G.D., Whincup, P.H., Rumley, A., Walker, M., Lennon, L., 2002. Physical activity and hemostatic and inflammatory variables in elderly men. Circulation 105, 1785 1790.

Wilmore, J.H., Stanforth, P.R., Domenick, M.A., Gagnon, J., Daw, E.W., Leon, A.S., Rao, D.C., Skinner, J.S., Bouchard, C., 1997. Reproducibility of anthropometric and body composition measurements: the HERITAGE Family Study. Int. J. Obes. Relat. Metab. Disord. 21, 297 303.

Wilson, A.G., di Giovine, F.S., Blakemore, A.I., Duff, G.W., 1992. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. Hum. Mol. Genet. 1, 353.

Wilson, A.G., Symons, J.A., McDowell, T.L., McDevitt, H.O., Duff, G.W., 1997. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc. Natl. Acad. Sci. U. S. A. 94, 3195 3199.

Winchester, E.C., Millwood, I.Y., Rand, L., Penny, M.A., Kessling, A.M., 2000. Association of the TNF-alpha-308 (G→A) polymorphism with self-reported history of childhood asthma. Hum. Genet. 107, 591 596.

You, T., Berman, D.M., Ryan, A.S., Nicklas, B.J., 2004. Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. J. Clin. Endocrinol. Metab. 89, 1739 1746.

Zhai, R., Jetten, M., Schins, R.P., Franssen, H., Borm, P.J., 1998. Polymorphisms in the promoter of the tumor necrosis factor-alpha gene in coal miners. Am. J. Ind. Med. 34, 318 324.