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Associations between inflammatory markers, candidate polymorphisms and physical performance in older Danish twins

Kristina Tiainen^{a,b,*}, Mikael Thinggaard^b, Marja Jylhä^a, Else Bladbjerg^c, Kaare Christensen^{b,d}, and Lene Christiansen^{b,d}

^aSchool of Health Sciences, University of Tampere, FI-33014 Tampere, Finland ^bThe Danish Twin Registry, and The Danish Aging Research Center, Epidemiology Unit, Institute of Public Health, University of Southern Denmark, DK-5000 Odense C, Denmark ^cUnit for Thrombosis Research, Institute of Public Health, University of Southern Denmark, DK-6700 Esbjerg, Denmark ^dDepartment of Clinical Biochemistry and Pharmacology, and Department of Clinical Genetics, Odense University Hospital, DK-5000 Odense, Denmark

Abstract

Inflammation may play an essential role in the decline of physical performance. In this study we investigated the associations between inflammatory markers, candidate polymorphisms and physical performance in elderly people. Plasma levels of TNF- α , IL-6, CRP, fibrinogen, sICAM-1 and candidate polymorphisms were measured in 600 twin individuals aged 73 years and older participating in the Longitudinal Study of Aging Danish Twins. Physical performance was assessed using a self-reported measure. The inclusion of twins allowed both traditional and within-twin-pair analysis which permitted control for shared environment and genetic factors. Higher levels of inflammatory markers were generally associated with a lower level of physical performance. The TNF α -238G/A polymorphism was significantly associated with physical performance in men, with A allele carriers having significantly better performance than GG homozygotes. However, this gene variation seems to have only a minor role in explaining the associations between the levels of inflammatory markers and physical performance. When using twin pair analysis to test whether genetic factors in general account for this association, results showed that the association between the level of fibrinogen and physical performance could be caused by genetic factors. Also the association between the level of TNF- α and physical performance in males could be caused by genetic factors. However, other gene variations than the candidate gene polymorphisms studied here seem to explain the major part of the genetic proportion of this association.

Keywords

Inflammation; Functional disability; Aging; Genetic; Twins

1. Introduction

The decline of physical performance is a multifactorial phenomenon which contains many different factors such as physiological age-related changes in muscle mass and muscle strength (Strotmeyer et al., 2009; Leveille, 2004), balance impairments and increased risk of

falling (Tiedemann et al., 2008), decreased resistance to fatigue (Vestergaard et al., 2009), and decreased physical activity (Buchman et al., 2007), and an increased number of chronic diseases (Hopman et al., 2009).

Elevated plasma/serum levels of inflammatory markers are known to be associated with many different diseases such as cardiovascular diseases (Danesh et al., 2008), diabetes mellitus (Dehghan et al., 2007), Alzheimer disease (Holmes et al., 2009), osteoporosis (McCormick, 2007), and also with mortality and shorter life expectancy (Jylhä et al., 2007; Bruunsgaard et al., 2003). The concentration of inflammatory markers also tends to increase with advancing age (Ferrucci et al., 2005).

Recent studies have demonstrated that inflammation may play an important role in the process of aging and in the development of functional limitations and disabilities (Ferrucci et al., 2002; Cesari et al., 2004; Visser et al., 2002; Tiainen et al., 2010; Brinkley et al., 2009). Elevated levels of inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP) have been associated with poor muscle strength (Ferrucci et al., 2002; Cesari et al., 2004; Brinkley et al., 2009; Stenholm et al., 2010) and decline in physical performance such as decreased walking speed and a longer time required for chair rise (Ferrucci et al., 2002; Cesari et al., 2004; Visser et al., 2002; Brinkley et al., 2009). Tumor necrosis factor- α (TNF- α) has a strong catabolic effect on muscle (Reid and Li, 2001) and it is associated with low muscle mass and muscle strength (Visser et al., 2002; Schaap et al., 2009; Bautmans et al., 2005) and with a steeper decline in muscle strength over time (Taekema et al., 2007). Elevated levels of fibrinogen are an important cardiovascular risk factor, but also increase risk of frailty (Walston et al., 2002).

Inflammatory marker production and the regulation of protein levels are believed to be partly genetically controlled (de Maat et al., 2004). However, results on the influence of commonly studied variations in relevant genes have been conflicting. In this study we evaluated the possible effects of some of the most repeatedly considered candidate polymorphisms of inflammatory marker genes for which associations with gene expression or protein levels of inflammatory markers have been demonstrated. We therefore included the IL6-174G/C polymorphism, which has been associated with concentrations of IL-6 (Fishman et al., 1998; Giacconi et al., 2004; Bonafe et al., 2001; Bruunsgaard et al., 2004); the CRP-1059G/C polymorphism, which has an effect on CRP levels (Vickers et al., 2002; Suk et al., 2005; Motoyama et al., 2009); the TNF α -238G/A polymorphism for which an influence on TNF- α gene expression has been shown (Huizinga et al., 1997) and the ICAM1-469K/E polymorphism suggested as a candidate variation within degenerative and inflammatory diseases (Amoli et al., 2002; Jiang et al., 2002). Finally, the fibrinogen-455G/A polymorphism has repeatedly been associated with plasma fibrinogen levels among middle-aged men and women (Tybjaerg-Hansen et al., 1997; van 't Hooft, et al., 1999).

Because these gene polymorphisms have been suggested to partly regulate the levels of inflammatory markers and since the levels of inflammatory markers are associated with physical performance, it may be hypothesized that these polymorphisms are also associated with variation in physical performance.

Accordingly, the aim of this study was to examine the associations between CRP, IL-6, TNF- α , soluble intercellular adhesion molecule-1 (sICAM-1), and fibrinogen and physical performance in old age using a cohort of older Danish twins, and to investigate if candidate gene polymorphisms (CRP-1059G/C, IL6-174C/T, TNF α -238G/A, ICAM1-469K/E, fibrinogen-455G/A) could in part explain this association.

2. Methods

2.1. Study population

The present study population consisted of 600 twins (200 male and 400 female) aged 73 to 95 years. From these individuals 132 monozygotic (MZ) twin pairs and 158 dizygotic (DZ) same-sex twin pairs were included in the genetic analyses. The twins participated in the second survey of the Longitudinal Study of Aging Danish Twins (LSADT) in 1997. LSADT has previously been described in detail (Christensen et al., 1999). In brief, LSADT is a cohort-sequential study launched in 1995 and repeated every second year up to 2005. Zygosity was determined through a questionnaire regarding the degree of similarity between twins in a pair. The zygosity classification was evaluated by comparison with DNA microsatellite analyses, and the misclassification rate was found to be less than 5% (Christiansen et al., 2003).

2.2. Measurement of physical performance

Physical performance was assessed using self-reported measures as part of the home-based 2-hour interview. The eleven-item measurement contains questions from relatively simple physical tasks to more demanding activities: 1) walking around in the house, 2) walking up and down the stairs, 3) walking up the stairs to the second floor, 4) getting outdoors, 5) walking 400m without resting, 6) doing any kind of light exercise, 7) doing any kind of hard exercise, 8) outdoor walking in fine weather, 9) outdoor walking in bad weather, 10) running 100 m and 11) carrying 5 kilos. At first all items were rated on a scale of 1 to 4 where 1=cannot do, 2=can do with aid or major difficulties, 3=can do with fatigue or minor difficulties, 4=can do without fatigue. Then the scale score (ADL strength score 1997) was calculated using a scale of 1 to 4, with higher scale scores indicating higher levels of physical performance. The calculations of the scores were described in detail in Christensen et al. (2000, 2002). This scale showed high internal consistency and stability and has been shown to provide a sensitive quantitative measure of physical ability in other studies of elderly Danes (Christensen et al., 2002).

2.3. Inflammatory markers and gene polymorphisms

Assessment of the plasma level of five inflammatory markers (CRP, IL-6, TNF- α , sICAM-1 and fibrinogen) and genotyping of commonly investigated polymorphisms in these genes were described in a previous study by de Maat et al. (2004). In short, the protein concentration of CRP was measured by a high-sensitivity in-house enzyme immunoassay using rabbit anti-human CRP IgG as the capture and tagging antibody (DAKO). Protein concentrations of IL-6, TNF- α , and sICAM-1 were determined using high-sensitivity human ELISAs (R&D Systems). The protein concentration of fibrinogen was determined using a nephelometric method (Dade Behring).

Genomic DNA samples were analyzed using polymerase chain reaction (PCR)-based methods, and the following polymorphisms were analyzed: fibrinogen β -455G/A (Thomas et al., 1994), CRP-1059G/C (Zee and Ridker, 2002), ICAM-1K/E469 (Nishimura et al., 2000), IL6-174C/T (Fishman et al., 1998), and TNF α -238G/A (Wennberg et al., 2002).

2.4. Statistical methods

Because all variables were not normally distributed, medians and interquartile ranges (IQR) were used to show the levels of inflammatory markers and physical performance. Differences between genders were analyzed using the non-parametric Wilcoxon rank-sum test.

The inclusion of twins allowed both traditional analysis and within-twin-pair analysis which controls for shared environment and genetic factors. The linear regression analysis was used to find the “crude” associations of physical performance with inflammatory markers and candidate polymorphisms. Because the biological characteristics of the twins within the pairs might be correlated, the analyses were performed using the cluster option in Stata, which only assumes independence between the pairs.

To further examine the associations between physical performance and the level of inflammatory markers and gene polymorphisms, respectively, within the pairs the fixed-effect analysis was used. In the fixed-effect analysis the effect of inflammatory markers and their gene polymorphisms on physical performance was calculated by canceling the effect of unknown shared factors (genetic or early family environmental influences) that might affect the level of physical performance (Fujiwara and Kawachi, 2009). If a significant association was observed in both MZ and DZ twin pairs, this provides evidence of unique environmental factors that probably affect both the inflammatory markers and the physical performance. If a significant association was observed only in DZ twins, the association between inflammatory markers and physical performance may be caused by genetic factors. If both the DZ and MZ twin pairs showed null associations, this suggests that the relation is caused by common environmental factors.

The difference in distribution of genotypes and the Hardy–Weinberg equilibrium were tested by the χ^2 -test. To test if the effects of inflammatory markers or gene polymorphisms on physical performance were different for men and women, gender interaction models were performed. In case of significant gender interactions, gender-specific analyses were performed in the linear regression analyses and in the fixed-effect analyses. Linear regression analyses and the fixed-effect analyses were adjusted for age and gender. The statistical analyses were done by using Stata 10.1 (STATA Corp., College Station, TX).

3. Results

Table 1 shows the plasma levels of inflammatory markers and the levels of physical performance of the participants. The level of physical performance in men was borderline higher ($p=0.052$) compared to women. Men had a significantly higher level of IL-6 ($p=0.034$) compared to women. Otherwise the levels of inflammatory markers did not differ significantly between the genders. We also checked the differences in levels of inflammatory marker and physical performance between the age groups of 73–79 and 80+. The level of physical performance was significantly lower in both men ($p=0.002$) and women ($p<0.001$) aged 80 years and older compared with 73–79-year-old men and women. Men and women of age 80+ furthermore had a significantly higher plasma level of TNF- α compared with 73–79-year-old men and women ($p=0.004$, $p<0.001$, respectively). In addition, the level of CRP in women aged 80 years and older was borderline lower ($p=0.048$) than in younger women. Otherwise the levels of inflammatory markers did not differ significantly between age groups.

The results of the age and gender adjusted linear regression analyses (Table 2) show that higher levels of IL-6, CRP and fibrinogen were significantly associated with a lower level of physical performance. Testing for gender interaction showed no significant gender differences in the analysis, although for men the association between the level of TNF- α and physical performance was statistically significant (Regr. coeff. $=-0.177$, $p=0.028$) compared to no significant association in women (Regr. coeff. $=-0.046$, $p=0.295$).

The distributions of the inflammatory marker gene polymorphisms in men and women are shown in Table 3. The distribution is in accordance with the predicted values assuming

Hardy–Weinberg equilibrium. In the further analysis two rare genotype groups were aggregated in fibrinogen-455 (GA, AA) and in TNF α -238 (GA, AA).

In the age and gender adjusted linear regression analysis for candidate gene polymorphisms and physical performance (Table 4), a significant effect of the TNF α -238G/A polymorphism was found. The A carriers (GA; AA) had a better physical performance level compared to the GG homozygotes. Testing for gender interaction revealed a significant gender difference in the association between the TNF α -238G/A polymorphism and the level of physical performance ($p=0.041$). In men the association between the TNF α -238G/A polymorphism and physical performance was more evident (Regr. coeff.=0.411, $p<0.001$) than in women (Regr. coeff.=0.076, $p=0.389$) where the association did not reach statistical significance. Otherwise the associations between the studied polymorphisms and physical performance were not statistically significant.

The results in Table 5 show the associations between the level of inflammatory markers and physical performance when adjusting for age, gender and each of the marker-specific polymorphisms investigated. Even though the TNF α -238 G/A polymorphism was associated with the level of physical performance (Table 4) this candidate gene polymorphism seems to play only a minor role in accounting for the association between the levels of inflammatory markers and physical performance. Only small changes can be seen in coefficients when comparing the age and gender adjusted models (Table 2) with the results in Table 5.

For testing whether genetic factors, in general, account for the association between the levels of inflammatory markers and physical performance the twin-fixed analyses were used. When performing the intra-pair analysis with all MZ and all DZ twin pairs the levels of fibrinogen were significantly associated with physical performance among DZ pairs but not among MZ pairs, suggesting that part of that association may be caused by genetic factors (Fig. 1). Furthermore, in males the level of TNF- α was significantly associated with physical performance only in DZ pairs (Regr. coeff.=−0.273, $p=0.032$ in DZ and Regr. coeff.=0.183, $p=0.252$ in MZ pairs) suggesting that part of the association between the level of TNF- α and physical performance could be caused by genetic factors. This was not seen in females.

4. Discussion

The results of the present study confirmed that the levels of inflammatory markers are associated with physical performance in men and women aged 73 to 95 years. Additionally, our study provides original contributions to the knowledge on this topic by examining whether a number of commonly investigated candidate gene polymorphisms are associated with physical performance. We found an interesting sex-specific association; however, the studied polymorphisms seem to play only a minor role in the association between the levels of inflammatory markers and physical performance. When using twin pair analysis to test whether genetic factors in general account for these associations, results indicated that a part of the apparent influence of the level of fibrinogen on physical performance in both genders, and the level of TNF- α on physical performance in men could be caused by genetic factors.

Our findings of a correlation between the levels of IL-6, CRP and fibrinogen with a lower level of physical performance are in line with the results of earlier studies which have shown that elevated levels of inflammatory markers are associated with both a lower level of physical performance and poor muscle strength (Ferrucci et al., 2002; Cesari et al., 2004; Visser et al., 2002; Tiainen et al., 2010; Brinkley et al., 2009). It is thus suggested that the elevated levels of inflammatory biomarkers have catabolic effects on muscle which can lead

to decreases in muscle mass and muscle strength that may also cause a decline in physical performance (Ferrucci et al., 2002).

Production and regulation of inflammatory marker levels in plasma or serum are in part genetically determined (Vickers et al., 2002; Suk et al., 2005). de Maat et al. (2004) demonstrated, with the same study population as was used in the present study, that genetic factors determine 26% of the variation in plasma levels of TNF- α , 21% in fibrinogen and 55% in sICAM-1. It could be hypothesized that some of the genetic variations commonly investigated in these genes may affect physical performance through variations in the levels of inflammatory markers.

Our results indicated that the TNF α -238G/A polymorphism was significantly associated with physical performance and the association was more evident in men. Thus, A carriers (GA; AA) had a better physical performance level compared to GG homozygotes. This is supported by the intra-pair analysis since the effect is only evident in male DZ twins. The twin analyses further indicated that, the association between the level of fibrinogen and physical performance may be caused by genetic factors. However, the results of the present study suggest that other variations than those studied here may explain the genetic proportion of the influence of TNF- α and fibrinogen levels on physical performance. Consistent with our results, in the study by Dato et al. (2010) the TNF α -238G/A and IL6-174C/T polymorphisms were not associated with hand grip strength or functional disability among Danish nonagenarians. Furthermore, Liu et al. (2008) found that the genetic variation in the TNF- α promoter made only a minor contribution to inter-individual variability in skeletal muscle phenotypes among men aged 20–97 years. In addition, they did not find any significant association between muscle phenotypes and the TNF α -238 polymorphism (Liu et al., 2008).

We additionally found that both the level of TNF- α and the TNF α -238G/A polymorphism appeared to be more important for physical performance in men than in women. One explanation for these findings could be based on the fact that TNF- α has been associated with muscle strength, and men generally have more muscles than women. Thus TNF- α has been suggested to be a mediator of sarcopenia, the age-related muscle atrophy. TNF- α has a catabolic effect on muscle and it may also have a role in the development of muscle wasting (Reid and Li, 2001). Furthermore, TNF- α alters the circulating levels of several hormones and stimulates the production of other catabolic cytokines. Muscle strength and physical performance have a strong relationship, as muscle strength is an important factor of independent daily living, and decreased muscle strength has been shown to increase risk of functional limitations and disabilities (Visser et al., 2005; Marsh et al., 2006). Hence TNF- α may influence the physical performance in men through an effect on muscle strength.

Our results also showed that the level of CRP and IL-6 were associated with physical performance, but the investigated polymorphisms in these genes were not. Based on our twin analysis it seems that direct genetic regulation is not an important factor in explaining the associations between these inflammatory markers and physical performance.

Aging and physical performance are complex phenomena which are affected by an interaction of genetic, environmental and lifestyle factors. In this study we hypothesized that the effect of candidate gene polymorphisms may work through the levels of inflammatory markers and in that way have an effect on physical performance. However, the results of our study showed that the effect of these particular candidate gene polymorphisms on physical performance is minor. Mainly other factors than gene variations accounted for the association between inflammatory markers and physical performance. Many chronic conditions raise the plasma concentration of inflammatory markers, but the levels also tend

to increase with aging (Stowe et al., 2010). Chronic low-grade inflammatory activity may have an important role in the process of aging and functional disabilities (Ferrucci et al., 2005; Ferrucci et al., 2002). It is difficult to say to what extent the elevation of the inflammatory marker levels are essential characteristics of the biological aging process or a consequence of distinct chronic disease.

The strength of the present study is that the inclusion of twins allowed us to use both traditional analysis and within-twin-pair analysis. However, a limited statistical power may be a problem in our twin pair analysis and may cause inconsistency of the results.

Although the participation rate was similar in men and women, especially the number of male participants in our study was small. The limited number of male twins is a consequence of the poorer survival rate of men compared to women, and this can also be seen in other studies among older people.

Finally, we did not perform a formal adjustment for multiple testing, since this study only included tests with a sound *a priori* hypothesis of effects from well known candidate polymorphisms. However, as the initial analyses performed only include five SNPs applying even the overly strict Bonferroni correction would leave still most of the findings significant.

In conclusion, the levels of inflammatory markers are associated with physical performance in men and women aged 73 to 95 years but only a minor part of this association was accounted for by the investigated genetic factors. Thus, gene variations other than the common polymorphisms studied here seem to explain the major part of the genetic proportion of the association between the levels of inflammatory markers and physical performance.

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Fig. 1.

Regression coefficients and 95% confidence intervals (CI) of inflammatory markers for all participants. The individual regression coefficients are results from Table 5 and the regression coefficients for dizygotic (DZ) and monozygotic (MZ) individuals are from twin fixed analysis. To fit the graphs into one figure the regression coefficients and confidence intervals are multiplied by 10 for IL-6, CRP and fibrinogen and by 100 for sICAM-1. TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; CRP, C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1.

Table 1
The levels of inflammatory markers and physical performance of the study population.

	Men						Women						All						
	73–79 years			80 years			73–79 years			80 years									
	n	Median (IQR)	p value	n	Median (IQR)		n	Median (IQR)	p value	n	Median (IQR)		Men	n	Median (IQR)	Women	n	Media (IQR)	p value
Age, years	151	76 (74–77)		49	83 (81–85)	<0.001	274	75 (74–78)		125	83 (81–86)	<0.001	200	77 (75–79)		399	77 (75–81)		0.354
ADL strength score 1997	151	3.50 (3.10–3.80)		49	3.30 (2.40–3.60)	0.002	274	3.50 (3.10–3.70)		125	2.90 (2.10–3.50)	<0.001	200	3.50 (3.00–3.75)		399	3.50 (2.70–3.70)		0.052
TNF-α, pg/ml	150	2.31 (1.90–2.78)		47	2.60 (2.19–3.11)	0.004	272	2.36 (1.97–2.84)		124	2.63 (2.19–3.37)	<0.001	200	2.37 (1.97–2.93)		396	2.45 (2.03–3.04)		0.254
IL-6, pg/ml	149	2.82 (1.94–4.51)		48	3.12 (2.12–4.95)	0.353	274	2.56 (1.66–3.97)		125	2.62 (1.93–4.85)	0.102	197	2.92 (1.98–4.57)		399	2.59 (1.71–4.16)		0.034
CRP, mg/l	147	1.56 (0.92–3.76)		48	1.77 (0.95–3.59)	0.493	270	1.80 (0.97–4.47)		121	1.28 (0.68–4.09)	0.048	197	1.61 (0.92–3.68)		391	1.63 (0.88–4.43)		0.934
Fibrinogen, μmol/l	151	12.60 (10.80–15.4)		49	13.20 (10.90–15.20)	0.490	275	12.50 (10.70–15.10)		125	12.70 (11.0–14.70)	0.747	195	12.75 (10.85–15.35)		400	12.60 (10.8–15.05)		0.814
sICAM-1, pg/ml	151	275.00 (243.00–322.00)		49	275.00 (248.00–327.00)	0.688	274	280.00 (240.00–316.00)		124	275.00 (241.50–318.50)	0.833	200	275 (243.50–322.50)		398	279 (241–318)		0.710

QOR, interquartile range; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; CRP, C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1. P values show the differences between age groups and between men and women (all).

Table 2

Age and gender adjusted associations between physical performance (ADL strength score 1997) and the levels of inflammatory markers.

	ADL strength score 1997			
	N	Regr. coeff.	95% CI	p value
TNF- α , pg/ml	592	-0.075	(-0.155, 0.005)	0.065
IL-6, pg/ml	595	-0.023	(-0.038, -0.008)	0.002
CRP, mg/l	585	-0.027	(-0.036, -0.017)	<0.001
Fibrinogen, μ mol/l	599	-0.043	(-0.061, -0.024)	<0.001
sICAM-1, pg/ml	597	-0.001	(-0.002, 0.0002)	0.139

95% CI, 95% confidence intervals; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; CRP, C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1.

Table 3

Genotype distribution of inflammatory marker gene polymorphisms.

	Men n (%)	Women n (%)
TNF α -238		
GG	110 (71.4)	211 (67.6)
GA	40 (26.0)	84 (26.9)
AA	4 (2.6)	17 (5.5)
IL6-174		
CC	37 (24.0)	105 (33.3)
CT	78 (50.7)	149 (47.3)
TT	39 (25.3)	61 (19.4)
CRP-1059		
GG	137 (90.7)	280 (89.2)
GE	14 (9.3)	34 (10.8)
EE	-	-
Fibrinogen-455		
GG	97 (63.0)	202 (64.1)
GA	53 (34.4)	101 (32.1)
AA	4 (2.6)	12 (3.8)
ICAM1-469		
KK	47 (30.5)	115 (36.5)
KE	73 (47.4)	144 (45.7)
EE	34 (22.1)	56 (17.8)

TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1.

Table 4

Age and gender adjusted associations between physical performance (ADL strength score 1997) and the gene polymorphisms of inflammatory markers.

	N	<u>ADL strength score 1997</u>		
		Regr. coeff.	95% CI	<i>p</i> value
TNFA-238	465			
GG		0 (ref.)		
GA/AA		0.181	(0.043, 0.320)	0.011
IL6-174	468			
CC		0 (ref.)		
CT		0.0002	(-0.141, 0.141)	0.998
TT		0.116	(-0.048, 0.279)	0.165
CRP-1059	464			
GG		0 (ref.)		
GE		0.047	(-0.165, 0.258)	0.663
EE		-	-	-
Fibrinogen-455	468			
GG		0 (ref.)		
GA/AA		0.024	(-0.118, 0.167)	0.736
ICAM1-469	468			
KK		0 (ref.)		
KE		0.131	(-0.022, 0.284)	0.093
EE		-0.08	(-0.299, 0.127)	0.425

TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1.

Table 5

Age and gender adjusted associations between physical performance (ADL strength score 1997) and the levels of inflammatory markers. Each individual inflammatory marker was adjusted for respective polymorphism.

	ADL strength score 1997			
	n	Regr. coeff.	95% CI	p value
TNF- α , pg/ml	451	-0.092	(-0.194, 0.009)	0.075
IL-6, pg/ml	455	-0.020	(-0.035, -0.006)	0.006
CRP, mg/l	444	-0.026	(-0.036, -0.016)	<0.001
Fibrinogen, μ mol/l	458	-0.044	(-0.066, -0.022)	<0.001
sICAM-1, pg/ml	457	-0.001	(-0.002, 0.001)	0.248

TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; CRP, C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1.