Joint effect of gene-physical activity and the interactions among CRP, TNF- α , and LTA polymorphisms on serum CRP, TNF- α levels, and handgrip strength in community-dwelling elders in Taiwan - TCHS-E

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Abstract This study assesses interactions of tumor necrosis factor α (TNF- α) gene polymorphisms with C-reactive protein (CRP) or lymphotoxin α (LTA) gene on serum CRP and TNF- α levels and handgrip strength. Eleven single nucleotide polymorphisms (SNPs), including rs2794520, rs1205, rs1130864, rs1800947, and rs3093059 in CRP; rs1799964, rs1800629, and rs3093662 in TNF- α ; and rs2239704, rs909253, and rs1041981 in LTA, were genotyped in 472 unrelated

elders (mean age 73.8 years). Among elders with TNF- α rs1799964 AA genotype, adjusted mean difference for handgrip strength decreased by -2.60 (-4.82 , -0.38) and -2.51 kg (-4.75 , -0.28) for LTA rs909253 and rs1041981 in women and by -2.39 kg (-3.98 , -0.81) for CRP rs3093059 in men. Among elders with TNF- α rs1799964 AA genotype, adjusted mean ratios for hs-CRP levels increased by 2.32 (1.38, 3.90) and 2.27 (1.35, 3.84) for both CRP rs909253 and rs1041981

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in women. The A-A-C LTA haplotype was associated with TNF- α levels that were 1.55 times higher than those of the C-G-A haplotype ($P = 0.005$). The joint effects of SNPs (the rs1800947 or rs3093059 of CRP, rs1799964 or rs1800629 of TNF- α , and rs909253 or rs1041981 of LTA) and physical inactivity appeared to have greater magnitude of decreased handgrip strength than main effects of these SNPs and physical inactivity. Our data showed that significant interactions of TNF- α rs1799964 and LTA rs909253 were observed. Moreover, joint effects of these CRP, TNF- α , and LTA risk alleles with physical inactivity in elders were observed, suggesting that physical activity may modulate effects of genotypes on handgrip strength.

Keywords CRP · TNF- α · LTA · SNP · Joint effect · Interaction · Handgrip strength

Introduction

Aging is related to systemic low-grade inflammation as well as to increased plasma levels of C-reactive protein (CRP) and tumor necrosis factor α (TNF- α) (Ballou et al. 1996; Bruunsgaard et al. 1999; Ferrucci et al. 2005; Krabbe et al. 2004). The elevation in the circulating level of inflammatory cytokines is also considered a predictor of sarcopenia (Ferrucci et al. 2002) and is associated with reduced muscle strength (Santos et al. 2011) and depression (Su 2012). Handgrip strength is usually regarded as an effective predictor of physical functional condition in elders (Rantanen et al. 1999), and previous studies have demonstrated that the heritability of handgrip strength is high (Frederiksen et al. 2002). This finding indicates that handgrip strength is a phenotype that can be used to identify the affected genes in the physical functional status of elders. Genetic factors may affect the physical function of the elderly because the hereditary factor of muscle strength may vary from 36 to 65 % (Arden et al. 1997; Carmelli et al. 2000; Frederiksen et al. 2002). Some association studies have suggested that inflammatory response is involved in the association between handgrip strength and gene polymorphisms (Arking et al. 2006; Dato et al. 2012).

CRP is a phylogenetically ancient regulator that is mapped in 1q23.2 (Floyd-Smith et al. 1986; Walsh et al. 1996). Several CRP gene polymorphisms are correlated with serum CRP level (Hage et al. 2007). Some studies suggest that 30–50 % of the phenotypic variation in

high-sensitivity CRP (hs-CRP) is determined genetically (de Maat et al. 2004; Pankow et al. 2001). Furthermore, the CRP polymorphisms rs1130864 (+1444C/T), rs1800947 (+1059G/C), and rs1205 (+1846C/T) affect CRP levels (Crawford et al. 2006; Frederiksen et al. 2002; Miller et al. 2005). Our previous study demonstrated that the single nucleotide polymorphisms (SNPs) rs2794520, rs1205, and rs3093059 of the CRP gene are associated with serum CRP level and handgrip strength in elders (Lin et al. 2014).

TNF- α and lymphotoxin α (LTA; also known as TNF- β) genes are members of the TNF superfamily. Both genes belong to a cluster of cytokines and are located next to each other in chromosome 6p21.3. This chromosome harbors the class III region of the major histocompatibility complex locus (Carroll et al. 1987). Various studies on the correlation between expression and disease have focused specifically on TNF. TNF- α is an early mediator in the acute phase response of the inflammation process, playing an important role in the initiation of the inflammatory cascade including the induction of liver-produced acute phase proteins such as CRP (Richards et al. 1995), and polymorphisms in the LTA gene are associated with the TNF- α production in the serum (Messer et al. 1991). Moreover, both TNF- α and LTA exert similar effects and share common receptors (Hehlhans et al. 2005). Three polymorphisms of the TNF- α gene are described in SNPs rs1800629 (−308G/A) and rs1799964 (−1031C/G) of the promoted region and in rs3093662 (+851A/G) of the first intron (Hajeer et al. 2000; Udalova et al. 1993). The TNF- α (−308) G/A polymorphism has been associated with altered promoter activity and TNF- α secretion (Wilson et al. 1997). The LTA rs909253 (+252) A/G polymorphism, which is located in the first intron of the LTA gene, is linked to high LTA expression (Messer et al. 1991). A previous study reported that the (−308) A and (+252) G alleles of the TNF- α SNP rs1800629 and the LTA SNP rs909253 are positively connected with inflammatory diseases such as asthma (Castro-Giner et al. 2008). Schaap et al. indicated that high levels of TNF- α are associated with significant declines in handgrip strength in men (Schaap et al. 2009). Liu et al. stated that genetic variations (−1031T/C and −863C/A) in the TNF promoter may contribute to inter-individual variability in muscle phenotypes in men (Liu et al. 2008).

Five tag-SNPs (rs2794520, rs1205, rs1130864, rs1800947, and rs3093059) in the CRP locus and six common genetic variants (rs1799964, rs1800629, and

rs3093662; rs2239704, rs909253, and rs1041981) in the TNF-LTA locus have been examined extensively as potential risk factors for diseases associated with inflammation, such as asthma (Castro-Giner et al. 2008), cancer (Huang et al. 2013), type 2 diabetes, and increased body mass index (BMI) (Qidwai et al. 2011). However, few studies explore the specific genes that may contribute to aging-associated decrease in physical performance, including handgrip strength. In particular, the genes involved in regulating the inflammation process are rarely investigated. The decline worsens with age and may induce individual variability in serum cytokine levels and in handgrip strength in older people. The effect of CRP polymorphisms on serum CRP level and handgrip strength has been explored (Lin et al. 2014), but the real effects of a single gene polymorphism on serum CRP and TNF- α levels, as well as on handgrip strength, may be masked by gene–gene and gene–environment interactions. Thus, the interaction of the genes on the same pathway should be analyzed to clarify actual influences on cytokine levels and handgrip strength. We hypothesize that the genetic variants of CRP, TNF- α , and LTA will be associated with gene–gene and gene–physical activity interactions, as well as the different influences of serum cytokine levels and handgrip strength in older men and women. The current study examines the association between the gene polymorphisms of inflammatory markers (CRP, TNF- α , and LTA) and either serum hs-CRP and TNF- α levels or handgrip strength among older men and women. We also analyze haplotypes and the potential main and interaction effects of these gene variants and physical activity.

Methods

Study subjects

This study included 472 unrelated elderly participants (221 women, 251 men; aged 65 years and over) of the Taichung Community Health Study for Elders. The design of the cross-sectional study and of the participant recruitment process has been described in detail in our previous publications (Lin et al. 2011; Lin et al. 2014). Eight subjects whose data were incomplete were eliminated from data analysis. All subjects provided their informed consent to participate in the study. The study was conducted in accordance with the requirements of

the Human Research Committee of China Medical University Hospital.

Measurements

The serum concentrations of hs-CRP and TNF- α were measured by nephelometry, which is a latex particle-enhanced immunoassay (TBA-200FR, Tokyo, Japan). The inter- and intra-assay coefficients of variation were <2.0 and <1.9 %, respectively. The low detection limit of the assay was 0.1 mg/L. The maximal isometric handgrip strength was determined in kilograms using a handheld dynamometer (TTM Dynamometer, Tsutsumi, Tokyo, Japan). The handgrip strength measurement process has been detailed previously (Lin et al. 2014).

A self-administered questionnaire was used to record the socio-demographic and lifestyle characteristics of each participant. The physical activity for leisure time of a participant was determined by the different kinds of physical activities each person participated in. The checklist of leisure-time activity contained 34 items, which could be classified into five types including ball games, martial arts, aerobic sports, dancing, and others. Each participant was asked to report his/her average time spent on one activity per occasion and the average number of the activity per week he/she spent performing the activity during the past 6 months. The subjects who reported that they currently participated in regular leisure-time activities for at least 30 min once per week during the preceding 6 months were classified as having regular physical activity. BMI (kg/m^2) was determined by dividing the weight of a subject in kilograms by the height in square meters. Blood samples were collected in the morning following an overnight fast of at least 12 h. These samples were analyzed within 4 h of collection.

SNP selection and genotyping

Eleven tag-SNPs containing five CRP tag-SNPs (rs2794520, rs1205, rs1130864, rs1800947, and rs3093059), three TNF- α tag-SNPs (rs1799964, rs1800629, and rs3093662), and three LTA tag-SNPs (rs2239704, rs909253, and rs1041981) were selected for genotyping based on the findings for the Han population from Beijing, China, in the International HapMap Project. Genomic DNA was extracted using a commercial kit (QIAamp DNA Blood Kit; Qiagen, Chatsworth,

CA, USA). All tag-SNPs were genotyped using the GoldenGate assay from Illumina, Inc. (San Diego, CA, USA). To improve the success rate of genotyping, we determined the risk of genotyping failure in advance. The genotypic distributions of each tag-SNP were estimated for Hardy–Weinberg equilibrium (HWE) in men and in women individually through an exact chi-square goodness of fit test. A threshold of $P < 0.05$ was applied to indicate departures from HWE.

Statistical analysis

The differences in demographic and lifestyle characteristics, including age, BMI, physical activity, smoking habits, and drinking habits between men and women were compared using either the two-sample t test (continuous variables) or the chi-square test (categorical variables). Exact HWE tests were conducted for each SNP using PLINK software. Gene–gene interactions or joint effects on handgrip strength, hs-CRP, and TNF- α level were assessed through linear regression analyses that were adjusted for age, BMI, physical activity, smoking habits, and drinking habits. The values of hs-CRP and TNF- α were natural log-transformed due to skewed distributions of the hs-CRP and TNF- α levels. Moreover, the geometric means and their standard deviations (SDs) were determined. The interactions between two SNPs and those between SNP and physical activity were examined by entering the product terms of either two SNPs or of SNP and physical activity with either the major allele of each SNP or no physical activity as the reference group. In addition, three dummy variables measured the effects of either of the two SNPs individually (or the influence of SNP only and that of physical activity only) and those of both SNPs combined (or the influence of both SNP and physical activity). The adjusted mean or geometric mean differences, or adjusted mean ratios, and the 95 % confidence intervals (CIs) of handgrip strength, hs-CRP, and TNF- α value were presented. Furthermore, the haplotypes in this sample were analyzed separately at an evaluation frequency >5 %. A two-sided $P < 0.05$ was considered statistically significant. The data were subject to stratified analyses by gender using Statistical Analysis System software (v9.3, SAS Institute Inc., Cary, NC, USA), PLINK (v1.07) (<http://pngu.mgh.harvard.edu/purcell/plink>) (Purcell et al. 2007), and Haploview (v4.2) (Barrett et al. 2005).

Results

The major characteristics of the subjects considered in this study are shown in Table 1. The mean age of the 472 community-dwelling elderly adults was 73.8 years (SD = 6.1 years). Among these participants, 53.2 % were men. The diagnosed diseases prevalently reported by participants were hypertension (47.4 %), diabetes (13.9 %), hyperlipidemia (25.7 %), cerebral vascular accidents (4.6 %), and cancer (5.5 %). Men displayed a high proportion of physical activity, smoking habits, alcohol drinking habits, hypertension, and diabetes. They also reported a high mean TNF- α level and level of handgrip strength. Women were also highly likely to suffer from hyperlipidemia. The values of the aforementioned variables differed significantly between men and women.

All 11 of the SNPs studied in the three genes were within the HWE ($P > 0.05$) (Table 2). The allele and genotype distributions for all polymorphisms did not vary in terms of sex ($P > 0.05$). The associations of the CRP and TNF/LTA polymorphisms with handgrip strength, serum hs-CRP, and TNF- α level were examined separately according to gender. The mean serum hs-CRP concentrations were higher in elders with the minor allele of the CRP gene SNPs rs2794520, rs1205, and rs3093059 than those with major allele of the corresponding SNPs; moreover, lower serum hs-CRP concentration was linked with greater number of A allele of LTA gene SNP rs2239704 (Table 2). The other polymorphisms were insignificantly associated with handgrip strength, serum hs-CRP, and TNF- α level. The Pearson correlation coefficients between handgrip strength and serum hs-CRP and TNF- α level (in natural log scale) were -0.16 ($P = 0.018$) and -0.13 ($P = 0.052$) in women, respectively. Similar trends were observed in men, which were -0.18 ($P = 0.005$) and -0.11 ($P = 0.089$). This indicated a modestly negative linear relationship between these two variables.

The findings from the multilocus CRP and TNF/LTA analyses are summarized in Table 3. The effects of interactions between the TNF- α SNP rs1799964 and LTA SNPs rs2239704, rs909253, and rs1041981 were significant for both handgrip strength ($P = 0.019$, 0.005, and 0.006, respectively) and serum hs-CRP level ($P = 0.023$, 0.001, and 0.001, respectively) in women when determined using a linear regression with multivariate adjustment. In addition, the interactions between the TNF- α SNP rs1800629 and the CRP SNP

Table 1 Characteristics of the study subjects according to gender

	Total (n = 472)	Women (n = 221, 46.8 %)	Men (n = 251, 53.2 %)	P value ^b
<i>Characteristic</i>				
Age (years)	73.8 ± 6.1	72.8 ± 5.5	74.7 ± 6.4	0.001
BMI (kg/m ²)	23.5 ± 3.2	23.5 ± 3.3	23.5 ± 3.2	0.829
Handgrip strength (kg)	27.6 ± 8.5	20.9 ± 5.4	33.4 ± 6.1	< .001
<i>Behaviors</i>				
Physical activity	376 (79.7)	167 (75.6)	209 (83.3)	0.038
Smoking	100 (21.2)	7 (3.2)	93 (37.1)	< .001
Alcohol drinking	94 (19.9)	14 (6.3)	80 (31.9)	< .001
<i>Biochemical marker</i>				
hs-CRP (mg/L) ^a	0.1 ± 3.2	0.1 ± 3.2	0.1 ± 3.2	0.884
TNF-α (pg/mL) ^a	1.6 ± 4.0	1.3 ± 4.0	1.8 ± 3.9	0.008
<i>Disease history</i>				
Hypertension	221 (47.4)	98 (45.2)	123 (49.4)	0.003
Diabetes	65 (13.9)	21 (9.6)	44 (17.8)	0.010
Hyperlipidemia	118 (25.7)	70 (32.1)	48 (19.8)	0.003
CVA	21 (4.6)	10 (4.6)	11 (4.5)	0.975
Cancer	25 (5.5)	13 (6.1)	12 (5.0)	0.615

Data were presented as mean ± SD for continuous variables or n (%) for categorical variables

BMI body mass index, hs-CRP high-sensitivity C-reactive protein, CVA cerebral vascular accident

^a Geometric mean was presented

^b Two-sample *t* test for continuous variables or chi-square test for categorical variables

rs2794520 or rs1205 were significant in women with respect to handgrip strength. Similarly, the interactions between the TNF-α SNP rs1799964 and the CRP SNP rs1130864 or rs3093059 were also significant in men in this respect. The interactions between the TNF-α SNP rs3093662 and the CRP SNP rs2794520 or rs1205 were significant on serum hs-CRP level in women. Similarly, the interactions between the TNF-α SNP rs3093662 and either the LTA SNP rs2239704 or the CRP SNP rs3093059 were significant in men in this sense.

To explore the significant interactions identified above further, we estimated the adjusted mean difference in handgrip strength or the adjusted mean ratio of hs-CRP levels for either LTA or CRP minor–major/minor–minor genotypes in comparison with the major–major genotype according to the genotype (minor–major/minor–minor and major–major) of the TNF-α SNPs (Fig. 1). Among elders with the GA/GG genotype of the TNF-α SNP rs1799964, the adjusted mean difference for handgrip strength decreased by −2.35 kg (95 % CI −4.41, −0.28) in women for the LTA SNP rs2239704 (Fig. 1A). However, this difference was not observed in elders with the AA genotype of the

TNF-α SNP rs1799964. On the contrary, among elders with TNF-α rs1799964 AA genotype, decreased adjusted mean differences for handgrip strength were observed for LTA rs909253 and rs1041981 by −2.60 kg (−4.82, −0.38) and −2.51 kg (−4.75, −0.28) in women and for CRP rs3093059 by −2.39 kg (−3.98, −0.81) in men, but no difference in elders with TNF-α rs1799964 GA/GG genotype. Among elders with TNF-α rs1800629 AG/AA genotype, increased adjusted mean differences for handgrip strength were observed for CRP rs2794520 by 3.58 kg (0.56, 6.60) and CRP rs1205 by 3.58 (0.55, 6.60) in women, but no difference in elders with TNF-α rs1800629 GG genotype.

Among elders with TNF-α rs1799964 AA genotype, increased adjusted mean ratios for serum hs-CRP levels were observed for both CRP rs909253 by 2.32 (1.38, 3.90) and CRP rs1041981 by 2.27 (1.35, 3.84) in women; but no difference in elders with TNF-α rs1800629 GA/GG genotype (Fig. 1B). Among elders with TNF-α rs3093662 GA/GG genotype, increased adjusted mean ratios for serum hs-CRP levels were observed for both CRP rs2794520 and rs1205 by 7.00 (1.70, 28.72) in women and CRP rs3093059 by 14.83 (1.38, 159.78)

Table 2 Genotype and allele distributions of study subjects as well as handgrip strength, serum hs-CRP, and TNF- α level distributions according to genotype status stratified by gender^a

Gene	SNP	Genotype or allele	Women (<i>n</i> = 221, 46.8 %)				Men (<i>n</i> = 251, 53.2 %)			
			Number (%)	Handgrip strength (kg)	Hs-CRP (mg/L) ^b	TNF- α (pg/mL) ^b	Number (%)	Handgrip strength (kg)	Hs-CRP (mg/L) ^b	TNF- α (pg/mL) ^b
CRP	rs2794520	TT	71 (32.1)	21.01 $\pm$ 5.94	0.08 $\pm$ 3.19 ^c	1.76 $\pm$ 3.49	78 (31.3)	33.20 $\pm$ 6.04	0.09 $\pm$ 2.69	2.02 $\pm$ 4.03
		CT	109 (49.3)	21.31 $\pm$ 4.83	0.10 $\pm$ 3.02 ^c	1.17 $\pm$ 4.02	119 (47.8)	33.88 $\pm$ 6.27	0.12 $\pm$ 3.47	1.87 $\pm$ 4.10
		CC	41 (18.6)	19.56 $\pm$ 5.84	0.19 $\pm$ 2.97	1.00 $\pm$ 4.44	52 (20.9)	32.47 $\pm$ 5.95	0.11 $\pm$ 3.30	1.53 $\pm$ 3.51
		C*	191 (43.2)				223 (44.8)			
	rs1205	TT	71 (32.3)	21.01 $\pm$ 5.94	0.08 $\pm$ 3.19 ^c	1.76 $\pm$ 3.49	78 (31.3)	33.20 $\pm$ 6.04	0.09 $\pm$ 2.69	2.02 $\pm$ 4.03
		CT	110 (50.0)	21.29 $\pm$ 4.81	0.10 $\pm$ 3.00 ^c	1.18 $\pm$ 4.00	118 (47.4)	34.11 $\pm$ 6.13	0.12 $\pm$ 3.49	1.83 $\pm$ 4.09
		CC	39 (17.7)	19.58 $\pm$ 5.99	0.20 $\pm$ 3.01	0.95 $\pm$ 4.51	53 (21.3)	32.32 $\pm$ 6.18	0.11 $\pm$ 3.25	1.47 $\pm$ 3.40
		C*	188 (42.7)				224 (45.0)			
	rs1130864	CC	194 (88.2)	20.93 $\pm$ 5.43	0.10 $\pm$ 3.21	1.26 $\pm$ 3.88	224 (91.1)	33.52 $\pm$ 6.06	0.10 $\pm$ 3.19	1.79 $\pm$ 3.94
		TC	26 (11.8)	20.63 $\pm$ 5.47	0.12 $\pm$ 3.06	1.53 $\pm$ 4.86	21 (8.5)	32.35 $\pm$ 7.15	0.12 $\pm$ 3.68	2.15 $\pm$ 3.80
TNF- α	rs1800947	TT	0 (0.0)	-	-	-	1 (0.4)	-	-	-
		T*	26 (5.9)				23 (4.7)			
		CC	181 (81.9)	20.64 $\pm$ 5.30	0.11 $\pm$ 3.05	1.26 $\pm$ 3.93	212 (84.5)	33.30 $\pm$ 6.21	0.11 $\pm$ 3.13	1.84 $\pm$ 4.06
		GC	37 (16.7)	22.24 $\pm$ 5.99	0.09 $\pm$ 3.64	1.46 $\pm$ 4.37	38 (15.1)	34.29 $\pm$ 5.69	0.09 $\pm$ 3.49	1.76 $\pm$ 3.40
	rs3093059	GG	3 (1.4)	19.45 $\pm$ 0.98	0.06 $\pm$ 8.28	1.52 $\pm$ 2.57	1 (0.4)	-	-	-
		G*	43 (9.7)				40 (8.0)			
		TT	142 (64.3)	21.41 $\pm$ 5.15	0.09 $\pm$ 3.35 ^c	1.36 $\pm$ 3.77	165 (65.7)	33.74 $\pm$ 6.15	0.10 $\pm$ 3.00	1.97 $\pm$ 4.01
		CT	68 (30.8)	19.91 $\pm$ 5.83	0.13 $\pm$ 2.62	1.17 $\pm$ 4.31	78 (31.1)	32.73 $\pm$ 6.35	0.12 $\pm$ 3.30	1.64 $\pm$ 3.89
		CC	11 (5.0)	20.28 $\pm$ 5.58	0.28 $\pm$ 2.57	1.34 $\pm$ 4.91	8 (3.2)	33.42 $\pm$ 2.72	0.16 $\pm$ 6.09	1.06 $\pm$ 2.73
		C*	90 (20.4)				94 (18.7)			
TNF- α	rs1799964	AA	139 (62.9)	21.19 $\pm$ 5.36	0.10 $\pm$ 3.03	1.22 $\pm$ 4.05	155 (62.0)	33.51 $\pm$ 6.47	0.11 $\pm$ 3.33	1.90 $\pm$ 3.99
		GA	73 (33.0)	20.17 $\pm$ 5.65	0.11 $\pm$ 3.66	1.44 $\pm$ 3.87	87 (34.8)	33.41 $\pm$ 5.77	0.09 $\pm$ 2.85	1.72 $\pm$ 3.86
		GG	9 (4.1)	22.00 $\pm$ 4.08	0.08 $\pm$ 1.73	1.33 $\pm$ 4.04	8 (3.2)	32.03 $\pm$ 3.45	0.18 $\pm$ 3.36	2.25 $\pm$ 3.57
		G*	91 (20.6)				103 (20.6)			
	rs1800629	GG	167 (77.0)	21.23 $\pm$ 4.91	0.10 $\pm$ 3.23	1.30 $\pm$ 4.01	206 (83.7)	33.21 $\pm$ 6.22	0.11 $\pm$ 3.27	1.85 $\pm$ 3.96
		AG	48 (22.1)	19.68 $\pm$ 6.97	0.12 $\pm$ 3.03	1.18 $\pm$ 4.15	40 (16.3)	34.71 $\pm$ 5.27	0.09 $\pm$ 2.60	1.73 $\pm$ 3.94
		AA	2 (0.9)	21.71 $\pm$ 0.12	0.05 $\pm$ 3.90	2.83 $\pm$ 1.22	0 (0.0)	-	-	-
		A*	52 (12.0)				40 (8.1)			
	rs3093662	AA	212 (95.9)	20.87 $\pm$ 5.49	0.10 $\pm$ 3.14	1.33 $\pm$ 3.99	241 (96.0)	33.49 $\pm$ 6.17	0.11 $\pm$ 3.17	1.85 $\pm$ 3.92
		GA	9 (4.1)	21.46 $\pm$ 3.44	0.14 $\pm$ 4.41	0.75 $\pm$ 3.41	10 (4.0)	31.58 $\pm$ 5.27	0.10 $\pm$ 3.61	1.38 $\pm$ 4.42

Table 2 (continued)

Gene	SNP	Genotype or allele	Women (<i>n</i> = 221, 46.8 %)				Men (<i>n</i> = 251, 53.2 %)			
			Number (%)	Handgrip strength (kg)	Hs-CRP (mg/L) ^b	TNF-α (pg/mL) ^b	Number (%)	Handgrip strength (kg)	Hs-CRP (mg/L) ^b	TNF-α (pg/mL) ^b
LTA	rs2239704	GG	0 (0.0)	-	-	-	0 (0.0)	-	-	-
		G*	9 (2.0)				10 (2.0)			
		CC	103 (46.6)	20.69 ± 6.13	0.11 ± 3.31 ^d	1.03 ± 3.78	111 (44.2)	33.46 ± 5.81	0.10 ± 3.12	1.84 ± 4.01
		AC	100 (45.3)	20.75 ± 4.56	0.12 ± 3.03 ^d	1.55 ± 4.10	107 (42.6)	33.25 ± 6.49	0.11 ± 3.30	1.64 ± 3.92
		AA	18 (8.1)	22.8 ± 5.40	0.05 ± 2.44	1.84 ± 3.69	33 (13.2)	33.83 ± 6.18	0.11 ± 3.10	2.47 ± 3.70
		A*	136 (30.8)				173 (34.5)			
		AA	60 (27.3)	20.60 ± 4.66	0.09 ± 3.37	1.85 ± 3.77	76 (30.3)	33.65 ± 5.45	0.10 ± 3.26	1.96 ± 3.64
		GA	110 (50)	21.35 ± 5.45	0.11 ± 2.96	1.21 ± 4.00	123 (49.0)	33.11 ± 6.65	0.11 ± 2.99	1.67 ± 4.03
		GG	50 (22.7)	20.13 ± 6.13	0.13 ± 3.38	0.98 ± 3.95	52 (20.7)	33.82 ± 5.87	0.10 ± 3.58	2.02 ± 4.20
		G*	210 (47.7)				227 (45.2)			
rs1041981		CC	59 (27.1)	20.63 ± 4.70	0.09 ± 3.40	1.84 ± 3.81	75 (30.2)	33.60 ± 5.48	0.10 ± 3.29	1.97 ± 3.67
		AC	108 (49.5)	21.46 ± 5.44	0.10 ± 2.98	1.19 ± 3.96	122 (49.2)	33.07 ± 6.67	0.11 ± 3.01	1.69 ± 4.03
		AA	51 (23.4)	20.24 ± 6.12	0.12 ± 3.44	0.98 ± 3.90	51 (20.6)	33.70 ± 5.86	0.10 ± 3.62	1.94 ± 4.13
		A*	210 (48.2)				224 (45.2)			

Data were presented as *n* (%) for genotypes and alleles

*Minor allele

^a All *P* > .05 from Hardy–Weinberg Equilibrium test^b Geometric mean was presented^c *P* value <0.05 for comparing with CC genotype using Bonferroni multiple comparison tests^d *P* value <0.05 for comparing with AA genotype using Bonferroni multiple comparison tests

Table 3 Significant gene–gene interaction on handgrip strength and hs-CRP

Gene 1	SNP 1	Gene 2	SNP 2	<i>P</i> for interaction	
				Handgrip strength	hs-CRP
<i>Women</i>					
TNF- α	rs1799964	LTA	rs2239704	0.019	0.023
TNF- α	rs1799964	LTA	rs909253	0.005	0.001
TNF- α	rs1799964	LTA	s1041981	0.006	0.001
TNF- α	rs1800629	CRP	rs2794520	0.004	
TNF- α	rs1800629	CRP	rs1205	0.005	
TNF- α	rs3093662	CRP	rs2794520		0.028
TNF- α	rs3093662	CRP	rs1205		0.028
<i>Men</i>					
TNF- α	rs3093662	LTA	rs2239704		0.039
TNF- α	rs3093662	CRP	rs3093059		0.047
TNF- α	rs1799964	CRP	rs1130864	0.048	
TNF- α	rs1799964	CRP	rs3093059	0.006	

and LTA rs2239704 by 0.23 (0.05, 0.98) in men. Similarly, in elders with TNF- α rs3093662 AA genotype, increased adjusted mean ratios for serum hs-CRP levels were observed for both CRP rs2794520 and rs1205 by 1.38 (1.01, 1.89) in women.

We conducted linear regression analyses on five SNP haplotypes in the CRP gene and on three such haplotypes in the TNF- α and LTA genes according to gender. Haplotypes with a frequency of <5 % were excluded from the analysis. In particular, the three and five haplotypes in the LTA and CRP genes of women, respectively, that are listed in Table 4 were included for analysis. The three haplotypes of CRP and LTA genes were highly prevalent, and the other two CRP haplotypes reported estimated frequencies of 9.7 and 5.9 %. Elderly women who carried the C–C–C–C–C CRP haplotype displayed significantly weaker handgrip strength ($\beta = -1.31$ kg; $P < 0.05$) and a higher hs-CRP level [$\exp(\beta) = 1.71$; $P < 0.001$] than those who bore the T–T–C–C–T haplotype. Moreover, participants who carried the C–C–T–C–T and C–C–C–C–T CRP haplotypes reported significantly higher hs-CRP levels than those who bore the T–T–C–C–T haplotype [$\exp(\beta) = 1.74$, $P = 0.017$; and 1.41, $P = 0.023$, respectively]. In addition, the A–A–C LTA haplotype was associated with a serum TNF- α level that is 1.55 times higher than that of the C–G–A haplotype ($P = 0.005$).

We evaluated the main and joint effects of gene and/or physical inactivity on handgrip strength using the

linear regression model. The joint effect of physical inactivity and the SNPs of CRP (rs1800947 and rs3093059), TNF- α (rs1799964 and rs1800629), and LTA (rs909253 and rs1041981) reduced handgrip strength in women compared with those who were physically active and possessed the major allele of the corresponding SNPs after adjusting for age BMI, smoking habits, and drinking habits (Table 5). The handgrip strength of men weakened in the same manner with the CRP SNP rs1130864 and the LTA SNP rs2239704. Therefore, physical inactivity weakens handgrip strength considerably when various SNPs ranging from -2.29 to -4.22 kg are considered. Moreover, physically inactive women with the CT or CC genotype of CRP rs3093059 had a significantly lower handgrip strength than those physically inactive women with the TT genotype of rs3093059 ($\beta = -3.55$ kg, $P = 0.007$).

Discussion

Inflammation may play a key role in the process of aging and in the development of functional disability. Among inflammatory biomarkers, circulatory concentrations of TNF- α were linearly associated with CRP in centenarians, thus indicating the connected activation of the entire inflammatory cascade in the oldest populations. In addition, these concentrations have been linked

A **B**

Fig. 1 **A** Adjusted mean differences of handgrip strength (kg) in gene 2 SNP stratified by gene 1 SNP for significant gene–gene interactions. **a** Adjusted mean difference of handgrip strength represents the adjusted mean for minor–major/minor–minor genotypes minus that for major–major genotype in gene 2 SNP stratified by gene 1 SNP. * $P < 0.05$; ** $P < 0.01$. **B** Adjusted mean ratio

of hs-CRP in gene 2 SNP stratified by gene 1 SNP for significant gene–gene interactions. **a** Adjusted mean ratio of hs-CRP represents the adjusted mean for minor–major/minor–minor genotypes divided by that for major–major genotype in gene 2 SNP stratified by gene 1 SNP. * $P < 0.05$; ** $P < 0.01$

to several age-related disorders (Bruunsgaard et al. 1999; Bruunsgaard et al. 2001; Kritchevsky et al. 2005). The association between elevated levels of inflammatory markers and decreased physical performance may be explained by the direct causal role of inflammatory markers in the age-associated decline of muscle mass and strength (Ferrucci et al. 2002). However, some studies reported no changes in serum

CRP and TNF- α levels with age (Fagiolo et al. 1993; Peterson et al. 1994). Therefore, our study examined the common variation in the genes that encode CRP, TNF- α , and LTA further. We also investigated their interactions with one another or with physical inactivity with respect to decreased handgrip strength simultaneously along with the CRP and TNF- α serum concentrations, especially those in both older men and women.

Table 4 The effect of CRP and LTA haplotypes on handgrip strength, hs-CRP, and TNF- α levels in women

SNP	Haplotype	Percentage of each haplotype	Handgrip strength		Ln hs-CRP ^a		Ln TNF- α ^a	
			β (SE) ^b	P value	β (SE) ^b	P value	β (SE) ^b	P value
CRP rs2794520-rs1205-rs1130864-rs1800947-rs3093059	T-T-C-C-T	47.1	Reference	–	Reference	–	Reference	–
	C-C-C-C-C	20.1	–1.31 (0.58)	0.023	0.54 (0.13)	1.71	–0.22 (0.17)	0.214
	T-T-C-G-T	9.7	0.19 (0.77)	0.808	0.19 (0.18)	1.21	0 (0.23)	0.995
	C-C-T-C-T	5.9	0.08 (1.03)	0.939	0.56 (0.23)	1.74	0.08 (0.31)	0.796
LTA rs2239704-rs909253-rs1041981	C-C-C-C-T	16.9	–0.43 (0.66)	0.515	0.34 (0.15)	1.41	–0.6 (0.2)	0.003
	C-G-A	48.0					Reference	–
	A-A-C	30.8					0.44 (0.16)	0.005
	C-A-C	21.3					0.22 (0.17)	0.209

 β estimated coefficient of regression model, SE standard error^aNatural log-transformation for high-sensitivity C-reactive protein and TNF- α ^bAdjustment of age, BMI, physical activity, smoking, and drinking^cExponential transformation for estimated coefficient, β

CRP is an important inflammatory biomarker and has been correlated with the development of functional disability in older people (Tiainen et al. 2010). Brinkley et al. revealed that high concentrations of CRP are correlated with worsened handgrip strength in individuals older than 55 years (Brinkley et al. 2009). Cesari et al. also noted that high levels of CRP are positively associated with poor physical performance and muscle strength in older persons (Cesari et al. 2004). In our study, we identified the effect of the interaction of CRP SNP rs2794520 or rs1205 with TNF- α SNP rs1800629 on handgrip strength in women, as well as that of the interaction of CRP SNP rs1130864 or rs3093059 with TNF- α SNP rs1799964 on this strength in men. Many studies revealed that individual SNPs are significantly associated with baseline CRP levels (Crawford et al. 2006; Kardys et al. 2006). Although a few of the individual SNP results are contradictory (Miller et al. 2005), our study demonstrated that the CRP SNPs rs2794520, rs1205, and rs3093059 are associated with high serum CRP level in elderly women. Furthermore, CRP SNP rs2794520 or rs1205 interacted with TNF- α SNP rs3093662 in women with respect to serum hs-CRP, whereas CRP SNP rs3093059 interacted with TNF- α SNP rs3093662 and LTA SNP rs2239704 in men.

We indicated previously that five SNPs in the studied CRP gene are arranged into the five haplotypes denoted for all subjects during haplotype analysis (Lin et al. 2014). In this study, during the investigation into the correlations of CRP, TNF- α , and LTA haplotypes with handgrip strength and serum CRP and TNF- α levels, we determined that some haplotypes affected serum CRP levels, TNF- α levels, and handgrip strength performance differently. In particular, the CRP haplotype C-C-C-C-C was significantly associated with increased serum CRP levels and decreased handgrip strength in women. The results for genetic polymorphisms that were correlated with high serum CRP levels and low handgrip strength at the single SNP level were similar at the haplotypic level.

The SNPs rs1800629 and rs1799964, which are located at the promoter region of TNF, may influence gene expression (Hajeer et al. 2001). They have been linked to autoimmune (Hollegaard et al. 2006) and age-related diseases (Bruunsgaard et al. 2004). SNP rs1800629 (TNF- α –308) has been

Table 5 Main and joint effect of SNPs and physical activity on handgrip strength

Gene	SNP	Combination	Women		Men	
			β (SE) ^a	<i>P</i> value	β (SE) ^a	<i>P</i> value
CRP	rs2794520	TT + PA	Reference	–	Reference	–
		CT/CC + PA	–0.60 (0.78)	0.444	–0.07 (0.75)	0.921
		TT + No PA	–4.22 (1.30)	0.001	–3.89 (1.34)	0.004
		CT/CC + No PA	–4.12 (1.05)	<.001	–3.13 (1.19)	0.009
	rs1205	TT + PA	Reference	–	Reference	–
		CT/CC + PA	–0.58 (0.78)	0.457	–0.03 (0.75)	0.973
		TT + No PA	–4.22 (1.30)	0.001	–3.88 (1.34)	0.004
		CT/CC + No PA	–4.12 (1.05)	<.001	–3.13 (1.19)	0.009
	rs1130864	CC + PA	Reference	–	Reference	–
		TC/TT + PA	0.06 (1.22)	0.963	–0.03 (1.31)	0.98
		CC + No PA	–3.97 (0.84)	<.001	–3.17 (0.91)	0.001
		TC/TT + No PA	–2.67 (1.59)	0.095	–4.38 (1.93)	0.024
	rs1800947	CC + PA	Reference	–	Reference	–
		GC/GG + PA	1.29 (0.88)	0.143	0.43 (0.96)	0.658
		CC + No PA	–3.30 (0.80)	<.001	–3.38 (0.93)	<.001
		GC/GG + No PA	–5.43 (2.13)	0.012	–3.21 (1.75)	0.069
	rs3093059	TT + PA	Reference	–	Reference	–
		CT/CC + PA	–1.22 (0.73)	0.094	–1.39 (0.70)	0.048
		TT + No PA	–2.83 (0.93) ^b	0.003	–4.09 (0.99)	<.001
		CT/CC + No PA	–6.38 (1.13) ^b	<.001	–3.37 (1.49)	0.024
LTA	rs2239704	CC + PA	Reference	–	Reference	–
		AC/AA + PA	–0.51 (0.72)	0.484	–0.45 (0.70)	0.519
		CC + No PA	–4.28 (1.06)	<.001	–3.21 (1.15)	0.005
		AC/AA + No PA	–3.7 (1.06)	0.001	–4.18 (1.23)	0.001
	rs909253	AA + PA	Reference	–	Reference	–
		GA/GG + PA	0.56 (0.80)	0.486	0.08 (0.72)	0.916
		AA + No PA	–2.29 (1.42)	0.109	–3.62 (1.72)	0.037
		GA/GG + No PA	–3.67 (1.03)	<.001	–3.28 (1.06)	0.002
	rs1041981	CC + PA	Reference	–	Reference	–
		AC/AA + PA	0.54 (0.81)	0.504	0.16 (0.73)	0.828
		CC + No PA	–2.32 (1.43)	0.107	–3.50 (1.73)	0.044
		AC/AA + No PA	–3.57 (1.06)	0.001	–3.14 (1.06)	0.003
TNF- α	rs1799964	AA + PA	Reference	–	Reference	–
		GA/GG + PA	–0.78 (0.74)	0.296	–0.16 (0.70)	0.822
		AA + No PA	–3.92 (0.96)	<.001	–3.86 (1.01)	<.001
		GA/GG + No PA	–4.19 (1.15)	<.001	–2.47 (1.48)	0.097
	rs1800629	GG + PA	Reference	–	Reference	–
		AG/AA + PA	0.24 (0.89)	0.791	1.73 (0.95)	0.069
		GG + No PA	–2.99 (0.89)	0.001	–3.46 (0.93)	<.001
		AG/AA + No PA	–5.41 (1.30)	<.001	–1.49 (1.65)	0.370
	rs3093662	AA + PA	Reference	–	Reference	–
		GA/GG + PA	–0.15 (1.67)	0.931	–2.49 (1.66)	0.135
		AA + No PA	–3.86 (0.77)	<.001	–3.58 (0.85)	<.001

Table 5 (continued)

Gene	SNP	Combination	Women		Men	
			β (SE) ^a	<i>P</i> value	β (SE) ^a	<i>P</i> value
		GA/GG + No PA	3.86 (4.61)	0.404	−1.59 (4.84)	0.742

β estimated coefficient of regression model, *SE* standard error

^a Adjustment of age, BMI, smoking, and drinking

^b Physically inactive women with the CT or CC genotype of CRP rs3093059 had a significantly lower handgrip strength than those physically inactive women with the TT genotype of rs3093059 (−3.55 kg (SE = 1.29), *P* = 0.007)

associated with blood TNF- α levels, inflammation, and susceptibility to several diseases (Abraham et al. 1999; Mira et al. 1999). In addition, reduced lean tissue mass has been associated with blood TNF- α levels in healthy older adults (Visser et al. 2002). Liu et al. indicated that rs1799964 (−1031C/G) variation in the TNF- α gene may contribute to inter-individual variation in muscle phenotypes in men (Liu et al. 2008). Nonetheless, these variants have not been associated with coronary artery disease and type 2 diabetes (Keso et al. 2001; Zeggini et al. 2005). Our study suggests that the three common genetic variants (SNPs rs1800629, rs1799964, and rs3093662) of the TNF- α gene are not related to the serum TNF- α levels in elderly men and women. Bruunsgaard et al. reported that the plasma levels of TNF- α are positively correlated with CRP in centenarians (Bruunsgaard et al. 1999). In the present study, the interaction between TNF- α SNP rs3093662 and CRP SNP rs2794520 or rs1205 in women significantly affected serum hs-CRP level but not TNF- α level, as did TNF- α SNP rs3093662 and CRP SNP rs3093059 in men. A possible reason why we failed to identify the main and interaction effects of CRP, TNF- α , and LTA genes on TNF- α level is that TNF- α is mainly produced in adipose tissue (Coppack 2001; Hotamisligil et al. 1993). Thus, it is difficult to detect in circulation given its short half-life unless large amounts are generated (Bruunsgaard 2006).

LTA is a crucial cytokine that has multiple functions in the immune system and in inflammatory reactions (Gray et al. 1984; McDevitt et al. 2002) as TNF- α . It also has an approximately 56 % sequence homology with TNF- α (Nedwin et al. 1985). Both are recognized by identical cellular TNF receptors and exert similar effects (Smith et al. 1994). The mutations of two LTA SNPs (rs1041981:

Thr26Asn, +804 C/A; rs2239704: +80 C/A) may regulate gene expression and the level of cytokine production (Messer et al. 1991; Tan et al. 2011). Furthermore, SNP rs1041981 is associated with the transcriptional regulation of LTA, which may activate lymphocytes and induce apoptosis (Ozaki et al. 2002). SNP rs2239704 can modulate both LTA levels and subsequent inflammatory response to pathogens (Knight et al. 2004). The (+252) G allele of SNP rs909253 may increase LTA production and TNF- α serum levels (Kohaar et al. 2009; Pociot et al. 1993). Our study also reports that the A–A–C LTA haplotype is associated with higher TNF- α serum levels than the C–G–A haplotype is. In addition, this research is the first to report the effect of the interaction between TNF- α and LTA polymorphisms on handgrip strength and serum hs-CRP levels in the elderly. Specifically, the significant interaction between the A allele of TNF- α SNP rs1799964 and either the G allele of LTA SNP rs909253 or the A allele of rs1041981 resulted in decreased handgrip strength and increased serum hs-CRP levels in elderly women (Fig. 1a, b).

The aging process and the health conditions related to age are complex phenotypes that are influenced by the interaction between genes and different environmental factors. Previous studies have shown that physical activity levels were inversely associated with inflammatory markers levels, including CRP (Abramson et al. 2002; Borodulin et al. 2006; Colbert et al. 2004; Geffken et al. 2001; Mattusch et al. 2000) and TNF- α (Gielen et al. 2003; Greiwe et al. 2001), and previous evidence suggested that physical activity increases muscle strength and physical performance (Ip et al. 2013; Tikkanen et al. 2012). Nevertheless, few studies explore the joint influence of CRP, TNF- α , and LTA gene variation and physical activity on handgrip strength. In

the present study, the main effect of the rs1800947 and rs3093059 of the CRP gene, the rs1799964 and rs1800629 of the TNF- α gene, and the rs909253 and rs1041981 of the LTA gene in women was reflected in decreased handgrip strength. The same effect was generated by the rs1130864 of the CRP gene and the rs2239704 of the LTA gene in men. The joint effect of the aforementioned SNPs and physical inactivity on handgrip strength reduced handgrip strength more than the main effect of SNP or of physical inactivity alone did. The potential explanation for the effect of physical inactivity and inflammation genes may be that the serum cytokines determined after physical activity induce an anti-inflammatory effect and may protect against CRP- and TNF- α -induced aging (Petersen et al. 2005). Thus, the evaluation of inflammatory biomarkers may be used to screen for low handgrip strength.

One limitation of the present study is that we only had the quantitative measurements for serum hs-CRP and TNF- α levels, and we did not have the measurement for serum TNF- β (LTA) level due to limited budget and the scarcity in reported associations between outcomes and TNF- β (LTA) in the literature. Thus, we cannot explore the relationship between these polymorphisms and TNF- β (LTA) in the present study.

Conclusion

In conclusion, our data suggest that the interaction of the A allele of the TNF- α SNP rs1799964 with either the G allele of LTA SNP rs909253 or the A allele of rs1041981 was associated with decreased handgrip strength and increased serum hs-CRP levels in older women. Moreover, the combined effects of these CRP, TNF- α , and LTA risk alleles and physical inactivity on decreased handgrip strength were identified, thereby suggesting that environmental factors may modulate the effects of genotypes on handgrip strength. These findings help delineate the genomic mechanisms underlying handgrip strength, serum hs-CRP, and TNF- α level and provide new insights for genetic screen testing. Health promotion programs focusing on physical activity should target at elders who are genetically predisposed to prevent low handgrip strength and further physical dysfunction.

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Compliance with ethical standards

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