Retrospective investigation of Interleukin *IL-1 and IL-6* genes polymorphism among elderly patients with sarcopenia in the Turkish population

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Genet. Mol. Res. 17 (1): gmr16039881

Received November 14, 2017

Accepted February 04, 2018

Published February 12, 2018

DOI http://dx.doi.org/10.4238/gmr16039881

ABSTRACT

Introduction: Sarcopenia, one of the geriatric syndromes, is defined as generalized and progressive reduction in skeletal muscle mass accompanied by loss of muscle strength and/or function. There is a paucity of research in the Turkish population about the effects of interleukin 1 (IL-1) and *IL-6* gene polymorphism on sarcopenia. The aim of this study was to evaluate the relationship between *IL-1* and *IL-6* gene polymorphism and sarcopenia in the older Turkish population.

Materials and Methods: The study included elderly residents of nursing homes who were 65 years or older had resided in a nursing home. Data regarding the patients' demographics, anthropometric measurements, muscle strength and physical performance were retrospectively examined. Sarcopenia screening was performed as specified in the European Union Geriatric Association's 2010 report entitled "The European Working Group on Sarcopenia in Older People". Fat-free mass (FFM) was compared to individuals in the general population between 18 and 45 years old with no illnesses or medication use (534 men, 180 women). Nursing home residents were divided into two groups: with or without sarcopenia. A blood sample was taken from each participant. DNA was obtained from peripheral blood and Interleukin 1 and 6 Gene Polymorphism was genotyped by polymerase chain reaction and restriction fragment length polymorphism method.

Results: A total of 149 elderly patients were included in the study. The overall rate of sarcopenia was determined as 42.2%. There was no statistically significant difference in sarcopenia based on IL-6, IL-1B31, and IL-1B511 genotype distributions and allele frequencies.

Conclusion: Our study demonstrates that sarcopenia in Turkish population is not influenced by *IL-1* and *IL-6* polymorphism.

Key words: Sarcopenia; Old age; Interleukin 1 and 6 genes

INTRODUCTION

The older population is growing in Turkey and worldwide. According to data from the Turkish Statistical Institute, the percentage of elderly the Turkish population is expected to reach 18.7% in 2050 and 20.7% in 2075. It is important to maintain functional independence along with this increase in the human lifespan (Raiche, et al. 2012). Walking speed is an easily applied test that indicates functional capacity in older people. Sarcopenia is known to be one of the factors that influence walking speed (Janssen, Baumgartner, et al. 2004; Fritz and Lusardi 2009; Cruz-Jentoft, et al. 2010).

Sarcopenia, one of the geriatric syndromes, is defined as generalized and progressive reduction in skeletal muscle mass accompanied by loss of muscle strength and/or function (Janssen, Baumgartner, et al. 2004). Although there is no definitive consensus on diagnostic criteria, various consensus reports have been published by different international groups. One such report published by the European Working Group on Sarcopenia in Older People (EWGSOP) defined the condition as low muscle mass and muscle function (reduced walking speed and/or muscle strength) (Cruz-Jentoft, et al. 2010).

The prevalence of sarcopenia varies depending on race, gender, and age (Abellan van Kan, et al. 2009). Sarcopenia further increases an aging individual's dependence, frequency of falls (Taaffe and Marcus 2000), mortality (Metter, et al. 2004; Rizzo, et al. 2008), and healthcare expenses (Janssen, Shepard, et al. 2004). Therefore, it is important to identify the factors that cause sarcopenia. Interleukin-6 (IL-6), interleukin-1 (IL1) are proinflammatory cytokines. After menopause or andropause, *IL-6* and *IL-1* levels are elevated, even in the absence of infection, trauma, or stress.

IL-6 and *IL-1* are potent mediators of inflammatory processes, and it has been proposed that the age-associated increase in *IL-6* and *IL-1* accounts for certain of the phenotypic changes of advanced age, particularly those that resemble chronic inflammatory disease (Ershler and Keller 2000; Benke, et al. 2011).There is a paucity of research in the Turkish population about the effects of interleukin 1 (IL-1) and *IL-6* gene polymorphism on sarcopenia. The aim of this study was to evaluate the relationship between *IL-1* and *IL-6* gene polymorphism and sarcopenia in the older Turkish population.

MATERIALS AND METHODS

The study included individuals aged 65 and above who were residents of a nursing home in Izmir for at least 1 month, were mobile, had full cognitive function, had no malignancy, and provided consent to participate in the study. The cognitive functions of the elderly were assessed by the Mini Mental (MM) test. This test was first described in the literature by Folstein et al. (Folstein, et al. 1975) .

Turkish validity and reliability of the test was done by Güngen et al. (Gungen, et al. 2002). The MM scores between 25-30 were normal and those below 25 were considered cognitive impairment. Exclusion criteria were age less than 65 years, nursing home residence less than 1-month, acute infections in the last month, active malignancy, inability to provide consent due to severe cognitive disorders, and immobility that prevented measurement of muscle mass, muscle strength, or walking speed.

Participants

Study participants were evaluated about demographic data such as age, gender, anthropometric measurements (height, weight, calf circumference, upper arm circumference, body mass index [kg/m^2]), muscle strength, and physical performance retrospectively. Sarcopenia screening was conducted according to the EWGSOP consensus report. Sarcopenia diagnosis was based on the presence of both low muscle mass and low muscle strength and/or physical performance (Cruz-Jentoft, et al. 2010). Physical performance was assessed with 6-meter walking speed, muscle strength was measured by handgrip dynamometer (Takei Physical Fitness Test), and fat-free body mass (FFM) by bioimpedance (Body Composition Analyzer SC-330).

A walking speed less than 1.0 m/s was considered low; muscle strength over 20 kg for women and 30 kg for men was accepted as normal. FFM was compared to community-dwelling adults 18-45 years old who had no diseases and were not using any medications (534 men, 180 women). Body surface area (BSA) was calculated

using the DuBois formula (BSA = $kg^{0.425} \times m^{0.725} \times 0.007184$). FFM was divided by BSA. Low muscle mass was considered below the 25th percentile of the control group (Castillo, et al. 2003).

Genetic Analysis

Blood samples (1 mL) were obtained from the study participants and DNA was isolated from a 200 µL aliquot for gene mutation analysis. Total genomic DNA was extracted from peripheral blood leukocytes using an automatic DNA isolation kit (QİAGEN, Hilden, Germany). Cells were disrupted with cell lysis solution to separate the cell membranes from the cell contents and proteinase K was used to remove all cellular and nuclear histone proteins and RNA. The DNA was precipitated onto the membrane with alcohol and washed to remove residual alcohol, protein, and membrane lipid contamination.

Elution buffer solution was used to elute 85-100% of the membrane-bound nucleic acids. DNA purity and concentration were assessed by measuring absorbance at 260 nm and 280 nm using the NanoDrop Spectrophotometer (Thermofisher). The DNA solution was diluted to obtain 50 ng/µL in a volume of 200 µL. DNA preparations with 260/280 absorbance ratios below 1.8 were considered contaminated. Two µl (100 ng) of DNA was separated by electrophoresis in 1% agarose gel.

The DNA was loaded on the gel with a standard DNA marker with known base number (Hae III Ferments) and imaged using Syngene ln Geneous (Syusome Genome) gel camera system. The previously verified DNA molecules were used in PCR reactions by combining 1 µL (100 ng) of genomic DNA, 2.5 µL Enhancer Buffer (20 mM Tris [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$), 0.5 µL of 0.2 mM dNTP mix, 1 µL forward primer (10 pmol/µL), 1 µL reverse primer (10 pmol/µL)(Invitrogen), 1.0 U PlatiniumTaq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) and deionized water to a total volume of 25 µL. The gradient program of the VERITI Gradient Thermal Cycler was used.

Positive PCR products were enzymatically purified using ExoSAP (Aversharun L, FeSeivee) reagent. Cycle sequencing by chain-termination method was performed on the purified amplification products using Big Dye cycle sequencing kit (Applied Biosciences). This step was conducted separately with both sense and antisense primers (bidirectional sequence).

The resulting amplicons were analyzed by agarose gel electrophoresis and positive amplicons were purified using the Big Dye XT purification kit (Applied Biosciences). Purified products were analyzed using capillary gel electrophoresis in an ABI3130xl automatic DNA analysis system. The resulting nucleotide sequences were compared with reference sequences (NM_005259) obtained from NCBI database in order to identify mutations or polymorphisms (NP005250). Distribution and allele frequency of identified mutations were determined to establish correlation with phenotypic features.

STATISTICAL ANALYSIS

This study included a patient group consisting of older adults with sarcopenia and a control group of older adults without sarcopenia. For intergroup comparisons of demographic data, numerical variables were expressed as mean ± standard deviation. Percentages were given for categorical variables, and chi-square analysis was used in statistical analyses. In order to determine the relation between the IL-6, IL-1B31, and IL-1B511 gene polymorphisms of sarcopenia patients and controls, allele frequencies were calculated, and the chi-square test was used for genes conforming to the Hardy-Weinberg proportions (HWP).

A multivariable logistic regression model was prepared with all possible gene combinations that may influence sarcopenia development via a compound effect of IL-6, IL-1B31, and IL-1B511 genotypes. Odds ratios with 95% confidence interval (CI) were calculated for significant correlations. All statistical analyses were done using IBM SPSS version 16. Statistical significance was accepted at $p \leq 0.05$. Approval to conduct the study was obtained from the Ataturk University Ethics Committee (decision dated 04/01/2018, ethics committee 01/9).

RESULTS

The 149 nursing home residents included in the study had a mean age of 76.56 ± 7.52 years, and 76 (51%) were female. The control group of healthy young adults (180 women with a mean age of 34.5 ± 8 years and 535 men with an average age of 35 ± 7 years) used as muscle mass reference had mean BSA values of 26.07 ± 2.05 kg/m^2 for females and 35 ± 2.46 kg/m^2 for males. BSA cut-off values calculated based on the 25th percentiles of

the reference group were 30.46 kg/m^2 for males and 24.67 kg/m^2 for females. According to these cut-off values, 63 (42.2%) of the nursing home residents had sarcopenia and 86 (57.8%) did not.

The prevalence of sarcopenia was significantly higher among males than females. About comorbid diseases, sarcopenia was significantly less common among participants with diabetes mellitus and obesity but was significantly more prevalent among patients with Parkinson's disease (Table 1).

Table 1. Distribution of demographic characteristics and comorbid diseases based on presence of sarcopenia

Variables		No Sarcopenia (n)	Sarcopenia (n)	p value
Demographic Characteristics				
Age (years, mean ± SD)		77.47 ± 7.19	75.32 ± 7.21	>0.05
Sex	Male	32 (36.4%)	44 (72.1%)	<0.001
	Female	56 (63.6%)	17 (27.9%)	
Marital status	Married	11 (12.5%)	4 (6.6%)	>0.05
	Single	77 (87.5%)	57 (93.4%)	
Education level	Illiterate	12 (13.6%)	11 (18.0%)	>0.05
	Literate	17 (19.3%)	7 (11.5%)	
	Elementary school	32 (36.4%)	23 (39.3%)	
	Middle school	5 (5.7%)	8 (13.1%)	
	High school	17 (19.3%)	10 (16.4%)	
	University/Graduate School	5 (5.7%)	1 (1.6%)	
Comorbid Diseases				
Ischemic heart disease		30 (33.7%)	15 (25.0%)	>0.05
Congestive heart failure		14 (15.7%)	4 (6.7%)	>0.05
Cerebrovascular disease		8 (9.0%)	4 (6.7%)	>0.05
Chronic lung disease		16 (18.0%)	15 (25.0%)	>0.05
Diabetes mellitus		25 (28.1%)	9 (15.0%)	0.046
Parkinson's disease		-	6 (10.0%)	0.004
Hypertension		63 (70.8%)	34 (56.7%)	>0.05
Osteoarthritis		26 (29.2%)	11 (18.3%)	>0.05
Dementia		23 (25.8%)	16 (26.7%)	>0.05
Depression		25 (28.1%)	14 (23.3%)	>0.05
Peripheral artery disease		1 (1.1%)	-	>0.05
Chronic liver disease		2 (2.2%)	1 (1.7%)	>0.05
Hip fracture		2 (2.2%)	1 (1.7%)	>0.05
Arrhythmia		4 (4.5%)	-	>0.05
Hyperlipidemia		11 (12.4%)	3 (5.0%)	>0.05
Obesity		71 (79.8%)	38 (63.3%)	0.022

Statistical analysis showed that the control group fit HWP about IL-1B511 and IL-1B31 polymorphism, but not *IL-6* polymorphism (p=0.591, 0.061 and 0.022, respectively). The patient group did not show HWP with regard to *IL-6* polymorphism but was in HWP with regard to IL-1B31 and IL-1B511 polymorphism (p=0.012, 0.206 and 0.92, respectively) (Table 2). *IL-6* allele frequencies could not be calculated because the control population deviated from HWP for *IL-6* genetic polymorphism. There was no statistically significant difference in sarcopenia based on IL-6, IL-1B31, and IL-1B511 genotype distributions and allele frequencies (Table 3).

To determine the joint effect of IL-6, IL-1B31, and IL-1B511 genotypes on sarcopenia development, we compared the relationship between genotype combinations in the patient and control groups of all three genes. To perform this analysis, we combined the CC, GC/TC, and GG/TT genotypes of the three genes. The *IL-6* CT, IL-1B31 TC, and IL-1B511GC combination was found to increase sarcopenia risk by 7.7-fold [CI 7.7 (0.284 – 46.364)] and the *IL-6* CT, IL-1B31 CC, and IL-1B511 GC combination by 7-fold [CI 7 (0.221 – 40.124)] (Table 4).

Table 2. Tests for Hardy-Weinberg proportions of IL-6, IL-1B31, and IL-1B511 genotypes in populations with and without sarcopenia

Variables	Genotypes	Observed value	Expected value	
***IL-6* Hardy-Weinberg proportions test**				
Sarcopenia	CC	8	13.54	X^2=8.22
	GC	41	29.92	P=0.004
	GG	11	16.54	
No Sarcopenia	CC	13	16.65	X^2=3.79
	GC	53	43.69	P=0.051
	GG	23	28.65	
IL-IB31 Hardy-Weinberg proportions test				
Sarcopenia	CC	6	8.82	X^2=2.37
	TC	34	28.37	P=0.123
	TT	20	22.82	
No Sarcopenia	CC	10	8.50	X^2=0.56
	TC	35	38.01	P=0.454
	TT	44	42.50	
IL-IB511 Hardy-Weinberg proportions test				
Sarcopenia	CC	25	22.2	X^2=2.29
	TC	23	28.59	P=0.130
	TT	12	9.20	
No Sarcopenia	CC	30	25.35	X^2=3.92
	TC	35	44.30	P=0.047
	TT	24	19.35	
	TT	24	19.35	

Table 3. Distribution of IL-6, IL-1B31, and IL-1B511 polymorphism allele and genotype frequencies according to presence of sarcopenia

	Sarcopenia	No Sarcopenia	p-value
IL-6			
C allele frequency	57	79	0.686
G allele frequency	63	99	
CC genotype frequency	8	13	0.448
CG genotype frequency	41	53	
GG genotype frequency	11	23	
IL-IB31			
C allele frequency	46	55	0.228
T allele frequency	74	123	
CC genotype frequency	6	10	0.102
TC genotype frequency	34	35	
TT genotype frequency	20	44	
IL-1B511			
CC genotype frequency	25	30	0.508
TC genotype frequency	23	35	
TT genotype frequency	12	24	

Table 4. Association between IL-6, IL-1B31, and IL-1B511 genotype frequencies and sarcopenia

IL-6	IL-IB31	IL-1B511	No Sarcopenia		Sarcopenia		Odds Ratio (confidence interval)		p-value
TT	TT	GG	10	11.24	1	1.67	1 (Reference)		
CT	TT	GG	3	3.37	3	5.00	10	(0.738 - 135.326)	0.08
TT	TC	GG	1	1.12	3	5.00	30	(1.410 - 638.150)	**0.02**
CC	TC	GG	6	6.74	1	1.67	1.6	(0.087 - 31.869)	0.73
TT	CC	GG	2	2.25	3	5.00	15	(0.982 - 228.896)	0.05
CT	CC	GG	1	1.12	2	3.33	-		1.00
CC	CC	GG	1	1.12	4	6.67	-		1.00
TT	TT	GC	3	3.37	5	8.33	6.6	(0.436 - 101.731)	0.17
CT	TT	GC	4	4.49	12	20.00	10	(0.838 - 119.314)	0.07
CC	TT	GC	1	1.12	2	3.33	-		1.00
CC	TT	GC	1	1.12	13	21.67	-		1.00
TT	TC	GC	14	15.73	3	5.00	3.5	(0.359 - 35.454)	0.28
CT	TC	GC	7	7.87	1	1.67	17.1	(1.794 - 163.806)	**0.01**
CC	TC	GC	2	2.25	1	1.67	-		1.00
TT	CC	GC	8	8.99	1	1.67	2.5	(0.190 - 32.802)	0.49
CT	CC	GC	10	11.24	1	1.67	13	1.4193 - 119.072	**0.02**
CC	CC	GC	3	3.37	3	5.00	10	(0.738 - 135.326)	0.08
TT	TT	CC	2	2.25	1	1.67	-		1.00
CT	TT	CC	4	4.49	1	1.67	5	(0.212 - 117.893)	0.32
TT	TC	CC	1	1.12	3	5.00	-		1.00
CT	TC	CC	2	2.25	3	5.00	-		1.00
CC	TC	CC	3	3.37	1	1.67	10	(0.317 - 315.278)	0.19
TT	CC	CC	10	11.24	3	5.00	15	(0.982 - 228.896)	0.05
CT	CC	CC	3	3.37	2	3.33	3.3	(0.156 - 70.905)	0.44

DISCUSSION

Sarcopenia is a geriatric syndrome characterized by progressive, generalized reduction in skeletal muscle mass with loss of muscle power and/or function (Abellan van Kan, et al. 2009). The factors causing sarcopenia to have been described in the literature. Hormonal, metabolic, and neurological factors, lack of physical activity, and immunosenescence are among the factors that lead to the development and progression of sarcopenia. Aging causes chronic inflammation and increased serum levels of proinflammatory cytokines such as IL-1, tumor necrosis factor alpha (TNF-alpha), and IL-6, and acute phase proteins such as C reactive protein (CRP)(Krabbe, et al. 2004; Maggio, et al. 2006).This also leads to increased mortality and morbidity in older people (Franceschi and Bonafe 2003; Krabbe, et al. 2004).

IL-6 is a cytokine which plays a role in proinflammatory response and in the neuroendocrine stress response (Ershler and Keller 2000; Krabbe, et al. 2004; Maggio, et al. 2006). Normally, *IL-6* is not found in the serum of young people unless trauma, infection, or another stressor is involved. Cell culture studies have revealed that *IL-6* levels in peripheral blood mononuclear cells increase with age (Forsey, et al. 2003; Krabbe, et al. 2004; Ferrucci, et al. 2005). Secretion of *IL-6* typically causes an inflammation cascade, leukocytosis, thrombocytosis, increased lymphocyte activation, and elevated acute phase proteins such as fibrinogen, C-reactive protein, and amyloid precursor protein (Ershler and Keller 2000). It has also been reported that increased serum levels of *IL-6* in older individuals causes cardiovascular and rheumatic diseases, sarcopenia, decreased functional capacity, and increased mortality (Krabbe, et al. 2004; Ferrucci, et al. 2005; Haddad, et al. 2005).

IL-6 secretion in older individuals is multifactorial and increases due to disease, reduced estrogen and testosterone levels, and the effect of genetic variants (Ershler and Keller 2000).The human *IL-6* gene is located on the short arm of chromosome 7 (7p21). *IL-6* secretion varies based on the secretion of its allelic variant (Pantsulaia, et al. 2002). It has been reported in the literature that *IL-6* plasma levels are influenced by the substitution of cytosine (C) for guanine (G) at 174.aa in young people (Fishman, et al. 1998; Olivieri, et al. 2002)older people(Bonafe, et al. 2001; Christiansen, et al. 2004)and centenarians(Bonafe, et al. 2001; Di Bona, et al. 2009). *In vitro* and *in vivo* studies have shown that the GG genotype increases *IL-6* levels (Fishman, et al. 1998; Bennermo, et al. 2004), while only a few studies have reported that the CC genotype increases *IL-6* (Jerrard-Dunne, et al. 2003; Haddy, et al. 2005)**.**

The *IL-1* gene is located on the long arm of chromosome 2 (2q13-21) and includes IL-1B and IL-A (Nicklin, et al. 2002). In our study including 149 nursing home residents, we did not find any statistically significant difference in sarcopenia regarding genotype distribution and allele frequencies of IL-6, IL-1B31, and IL-1B511. There is limited research in the literature examining the effects of *IL-6* polymorphism in sarcopenia. Roth et al.(Roth, et al. 2003) evaluated the association between IL-6174 C/G gene polymorphism and muscle mass and muscle strength in a study including 110 males and 132 females ranging in age from 21 to 91 years. The study revealed a correlation between *IL-6* gene polymorphism and lean muscle mass in men, but not in women (Roth, et al. 2003). In another study by Waltson et al.(Walston, et al. 2005) including a total of 463 people between the ages of 70-79 years, *IL-6* level and polymorphism was not associated with muscle strength and frailty. Pereira et al. (Pereira, et al. 2011) also determined that *IL-6* polymorphism did not have an effect on muscle strength in their study of 199 elderly female nursing home residents and community-dwelling individuals aged 65-81 years. In our study, sarcopenia screening was performed according to the EWGSOP diagnostic criteria and no correlation was found between *IL-6* polymorphism and sarcopenia.

CONCLUSION

Previous studies have shown that changes in *IL-1* gene are correlated with lupus, vasculitis, renal failure, cognitive dysfunctions, and cardiovascular diseases (Wetmore, et al. 2005; Benke, et al. 2011). However, the effect of *IL-1* gene polymorphism on sarcopenia has not been investigated. In the present study, we did not find any correlation between sarcopenia and *IL-1* gene polymorphism. In this respect, our study is important for providing the first data on this topic. The main limitation of this study is the inclusion of elderly nursing home residents from only one city. Prospective studies including larger numbers of older adults are needed. Secondly, the correlation between sarcopenia and *IL-1* and *IL-6* gene polymorphism was examined, but serum levels of these cytokines were not evaluated. However, strength of our study is that it is the first in our country to investigate the association between *IL-1* and *IL-6* gene polymorphism and sarcopenia. Our study demonstrates that sarcopenia in Turkish population is not influenced by *IL-1* and *IL-6* polymorphism.

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