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ORIGINAL ARTICLE

VNTR polymorphisms of the *IL-4* and *IL-1RN* genes and their relationship with frailty syndrome in Mexican community-dwelling elderly

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Abstract Inflammation is a key event that is closely associated with the pathophysiology of frailty. The relationship of genetic polymorphisms into inflammatory cytokines with frailty remains poorly understood. The aim of this study was to investigate the association between VNTR polymorphisms of the *IL-4* and *IL-1RN* genes with the risk of frailty. We included a sample of 630 community-dwelling elderly aged 70 and older. Both *IL-4* and *IL-1RN* VNTR polymorphisms were genotyped by the polymerase chain reaction (PCR) method. Mean age was 77.7 years (SD = 6.0) and 52.5 % were women. The participants classified as frail were more likely to be older, had lower MMSE score ($p < 0.001$), and had more disability for IADL ($p < 0.001$) and ADL ($p < 0.001$). Genotypic and allelic frequencies for the *IL-4* VNTR polymorphism did not show significant differences between study groups ($p > 0.05$). However, we just observed a significant difference in the allelic frequencies for the A2 allele of the *IL-1RN* VNTR polymorphism between frail and nonfrail groups (OR 1.84, 95 % CI 1.08–3.12, $p = 0.02$). In addition, we analyzed the combined effect of the *IL-4* and *IL-1RN* VNTR polymorphisms and their possible association with frailty, where the combined *IL-4*^{low}–*IL-1Ra*^{high} genotype was identified as a marker of risk to frailty syndrome (OR 7.86, 95 % CI 1.83–33.69, $p = 0.006$). Our results suggest that both A2 allele and the combined *IL-4*^{low}–*IL-1Ra*^{high} genotype might be genetic markers of susceptibility to frailty in Mexican elderly.

Keywords Frailty · *IL-4* · *IL-1RN* · VNTR polymorphism · Genetic susceptibility · Older adults

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Introduction

Frailty has been defined as a multidimensional syndrome characterized by multisystem dysregulations, leading to a loss of dynamic homeostasis, decreased physiologic reserve, and increased vulnerability for subsequent morbidity and mortality [1]. Chronic low-grade inflammation and immune activation are closely involved in the underlying mechanism that contributes to frailty, and mounting evidence from in vivo studies has shown that inflammatory cytokines play a crucial role in its development [2–5].

It has been proposed that genetic variation in pro- or anti-inflammatory cytokines is capable of modulating the susceptibility, severity, and clinical outcome of age-related diseases [6, 7]. Even if studies have shown that inflammatory pathway activation is a heritable trait, few

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studies have evaluated the genetic risk for frailty [8] and, to the best of our knowledge, no reports have been published regarding the role of the polymorphisms into immune system genes in frailty syndrome. In this regard, the interleukin-4 (IL-4) and interleukin-1 receptor antagonist (IL-1Ra) are two cytokines, which could be associated with the frailty syndrome due to their biological importance.

IL-4 is a potent anti-inflammatory cytokine, characterized by inducing the synthesis and release of other anti-inflammatory molecules, such as interleukin-10 (IL-10) and IL-1Ra [9]. IL-4 also inhibits the secretion of the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 (IL-1) [10], and it is also able to induce the synthesis and release of anti-inflammatory factors such as the IL-1Ra. The gene encoding IL-4 is located in the chromosome 5q31.1 [11, 12] and there is a 70 bp variable number of tandem repeats (VNTR) polymorphism in its third intron which could change the expression level of *IL-4* gene [13, 14], with the RP1 allele enhancing IL-4 expression compared with the RP2 allele [13, 15, 16]; moreover, this polymorphism has been reported to be associated with several inflammatory diseases [9, 12].

IL-1Ra is another important immunologic regulator that competes with other IL-1 family members for the IL-1 receptor and acting as a negative regulator with anti-inflammatory effects [17, 18]. The gene encoding IL-1Ra (*IL-1RN*) is located in the chromosome 2q14 [19, 20], and there is a 86 bp VNTR polymorphism in its second intron [21, 22]. The allele 2 of this VNTR has been widely shown to be associated with a number of inflammatory diseases [22], and has been reported to be associated with an increase of the IL-1β production in vitro [20]. In addition, several reports have provided evidence that this polymorphism plays a role in the susceptibility and the severity of a variety of disorders, including age-related diseases [7].

Disturbance of the anti-inflammatory response might be a critical component of the chronic low-grade inflammation found in the frailty. Therefore, the main hypothesis in this study was that the VNTR polymorphisms of the *IL-4* and *IL-1RN* genes could be used as biomarkers of inflammation to predict the risk of frailty among Mexican elderly population. Until now, the association between the VNTR polymorphisms into the *IL-4* and *IL-1RN* genes as well as their combined effect and the frailty syndrome in Mexican elderly is unknown. Therefore, the aim of this study was to evaluate the relationship of the VNTR polymorphisms of the *IL-4* and *IL-1RN* genes with susceptibility to frailty in older Mexican adults.

Materials and methods

Study population

The sample of this cross-sectional study is a subset of individuals who participated in the Mexican Study of Nutritional and Psychosocial Markers of Frailty (the "Coyoacán cohort"), a prospective cohort study aiming to evaluate the nutritional and psychosocial determinants of frailty among Mexican community-dwelling elderly. A detailed description of the participants and methodology of this study have been reported elsewhere [23]. Briefly, participants were identified through the listing of a government program which includes 95 % of community-dwelling elderly subjects aged 70 or older living in Mexico City, and which constituted the study's sampling frame. Recruitment was drawn from a random sample procedure, stratified by age and sex and confined to Coyoacán, one of the 16 districts of Mexico City. Among the contacted subjects, the acceptance rate was 86.9 % and a total of 1124 participants were finally included in the study. Baseline data were collected in two phases. During the first one, participants were examined at home, and data were collected through a face-to-face interview using a standardized questionnaire. A wide range of information was collected during this phase including socio-demographic factors as well as health issues. In the second phase, an interdisciplinary team constituted by physicians, nurses, nutritionists and dentists evaluated participants. The subjects underwent a comprehensive geriatric assessment including functional status, co-morbidity, pharmacological treatments, physical performance, nutritional state, oral health, arterial tension, and anthropometry. Blood samples were drawn from a subset of the total population (84 %, $n = 945$) and several determinations were made; about 30 % of this subset was randomly selected for the determination of 25(OH)-vitamin D levels.

Definition of frailty

Frailty was defined as proposed by Fried and colleagues in the Cardiovascular Health Study [24]. All five components from the original phenotype were retained, but the metrics used to characterize each criterion were slightly different and defined as follows: (1) Weight loss was defined as self-reported unintentional weight loss of 5 kg or more within the past year. (2) Exhaustion or fatigue (low energy) was indicated by self-response of two questions from the Center for Epidemiological Studies-Depression Scale (CES-D): "I felt that everything I did was an effort" and "I could not get going" [25]. Participants were asked: "How often, in

the last week, did you feel this way?" Possible answers were 0 = rarely or none of the time; 1 = some or a little of the time; 2 = a moderate amount of the time and 3 = most of the time. Participants answering "2" or "3" to either of these questions were considered as frail by exhaustion. (3) Low physical activity was defined by the Spanish version of the Physical Activity Scale for the Elderly (PASE) [26]. Participants who scored in the lowest quintile, adjusted by sex, were considered frail for this component. (4) Slowness was considered to be present if subjects gave two positive answers to the questions: "Does your actual health limit you to climb a single flight of stairs?" and "Does your actual health limit you to walk a 100 m block?". (5) Weakness was defined by a positive answer to the question: "Because of your current health status, do you have any difficulties lifting a 5 kg weight, such as a heavy grocery bag?". For the purpose of this study, the participants were considered to be "frail" if they met three or more of the five frailty criteria, "prefrail" if they fulfilled one or two frailty criteria or "nonfrail" if none.

Genomic DNA (gDNA) extraction and genotyping

For the genetic analysis, gDNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (QIAamp®DNA, Qiagen). The VNTR polymorphisms of the *IL-4* and *IL-1RN* genes were analyzed by polymerase chain reaction (PCR) method as follows:

- To amplify the 70 bp VNTR region of *IL-4* gene intron 3, we used the following primers: forward (5′-TAGGCTGAAAGGGGGAAAGC-3′) and reverse (5′-CTGTTCACCTCAACTGCTCC-3′) (Invitrogen™ life technologies, Carlsbad, CA, USA) [27]. The PCR was performed in a 25 µL final volume that contained 100 ng of gDNA, 1 µM of each primer, 0.5 unit of *Taq* DNA polymerase, PCR buffer 1X, 2 mM of $MgCl_2$ and 0.2 mM of each dNTPs (Invitrogen™ life technologies), according to the following protocol: initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. Finally, the PCR products were separated by electrophoresis on a 2 % agarose gel (Invitrogen™ life technologies) and visualized by ethidium bromide staining (Fig. 1a).
- The region that contains the VNTR of 86 bp within *IL-1RN* intron 2 was amplified using the following oligonucleotides: forward (5′-CTCAGCAACACTCCT AT-3′) and reverse (5′-TCCTGGTCTGCAGGTAA-3′) (Invitrogen™ life technologies) [28]. The PCR was conducted in a total volume of 25 µL, containing 100 ng of gDNA, 1 µM of each primer, 0.5 units of *Taq* DNA polymerase, PCR buffer 1X, 2 mM of MgCl2 and 0.2 mM of each dNTPs (InvitrogenTM life technologies), according to the following protocol: initial denaturation at 93 °C for 3 min, 35 cycles of denaturation at 93 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 3 min. The PCR products were separated by electrophoresis on a 2 % agarose gel (Invitrogen™ life technologies) and visualized by ethidium bromide staining (Fig. 1b).

Covariates

Socio-demographic variables included in this study were: age (years), sex, educational level (years) and self-reported socio-economic status (excellent, very good, good, regular and poor). The presence of seven chronic diseases was summed up in a score ranging from 0 to 7, where a higher score indicates more chronic diseases (myocardial infarction, stroke, cancer, hypertension, diabetes, dyslipidemia, and thyroid disease). Cognitive function was evaluated through the Mini-Mental State Examination (MMSE), with a score ranging from 0 to 30 and where lower scores indicate poorer cognitive performance [29]. Two domains of disability were investigated, disability for basic (ADL) and instrumental activities of daily living (IADL). For ADL, subjects were asked about their ability to carry out the following tasks without help: bathing, dressing, toileting, transferring, continence, and feeding themselves [30]. Whereas for IADL, participants reported their ability to perform the following eight activities: using the telephone, shopping, grooming, housekeeping, doing laundry, using transportation, handling medications, and handling finances [31]. For each domain of disability, if participants indicated that they were unable to perform at least one of the activities without help, they were considered as having ADL or IADL disability, respectively.

Sample

The sample was taken from the Coyoacan Cohort Study, and was estimated using a random sample procedure to ensure a sample size with a prevalence of at least 14 % of frailty among participants with $\alpha = 5$ % and $\beta = 20$ % ($n = 1294$). However, only 1124 elders were interviewed, of which 743 accepted being subject to the biological sampling. Finally, for the present study, of the 743 subjects with biological sample, 113 were excluded for incomplete frailty data. Therefore, we only included information from

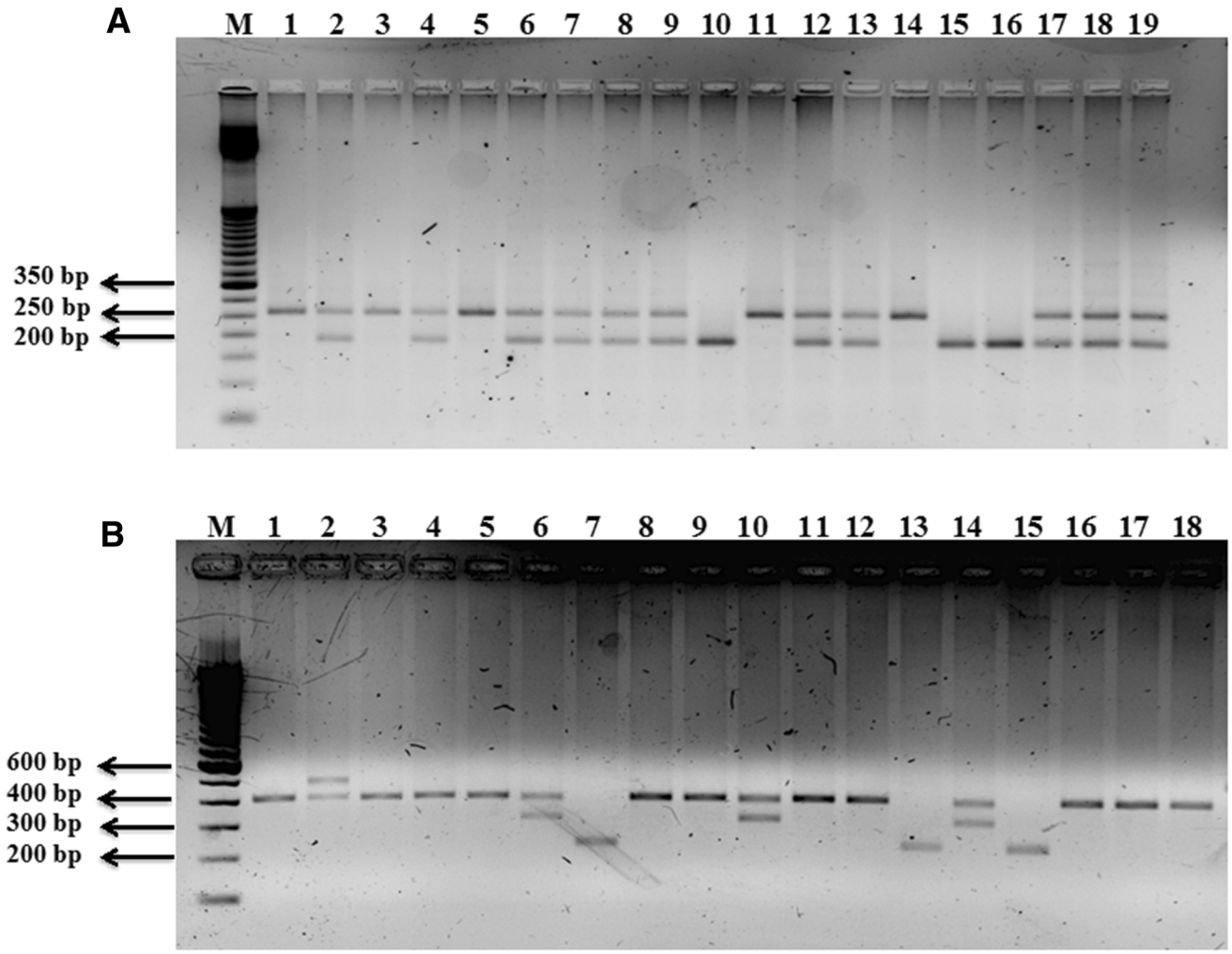

Fig. 1 Identification of the *IL-4* and *IL-1RN* VNTR polymorphisms. **a** Agarose gel electrophoresis showing the PCR products of 70 bp for the *IL-4* VNTR polymorphism. *M* 50 bp DNA ladder; *lanes 10*, *15* and *16* RP1/RP1 genotype; *lanes 2*, *4*, *6–9*, *12*, *13*, *17–19* RP1/RP2 genotype; *lanes 1*, *3*, *5*, *11* and *14* RP2/RP2 genotype. **b** Agarose gel electrophoresis showing the PCR products of 86 bp for the *IL-1RN* VNTR polymorphism. *M* 100 bp DNA ladder; *lanes 1*, *3–5*, *8*, *9*, *11*, *12* and *16–18* A1/A1 genotype; *lane 2* A1/A3 genotype; *lanes 6*, *10* and *14* A1/A4 genotype; *lanes 7*, *13* and *15* A2/A2 genotype

331 (52.5 %) women and 299 (47.5 %) men for the final statistical analysis.

Statistical analysis

Variables were described using frequencies and proportions or arithmetic means and standard deviations (SD) when appropriate. The statistical analysis was carried out using SPSS statistical package version 17.0, Excel 2010 and Genetic Data Analysis Computer Program for the analysis of allelic data [32]. The χ^2 test (or Fisher's exact test when applicable) was used to compare discrete variables and to test the Hardy–Weinberg equilibrium. For other comparisons, we used one-way ANOVA test. The significance level was a p value of <0.05. Odds ratio (OR) was the association measure.

Results

The study sample included 630 participants. Table 1 shows the socio-demographic characteristics and health status of participants according to their frailty status. Mean age was 77.7 years (SD = 6.0) and 52.5 % were women. The participants classified as frail were more likely to be older, had lower MMSE score ($p < 0.001$), and had more disability for IADL ($p < 0.001$) and ADL ($p < 0.001$) in comparison with nonfrail subjects. There were no differences between the groups regarding educational level, self-report economic situation, and co-morbidity score.

The genotypic and allelic frequencies of the VNTR polymorphisms into both *IL-4* and *IL-1RN* genes are shown in Tables 2 and 3, respectively. The study population was found to be in Hardy–Weinberg equilibrium for both

Table 1 Socio-demographic and clinical characteristics of participants according to their frailty status

Variable	Status of frailty				*p*
	Overall ($n = 630$)	Nonfrail ($n = 320$)	Prefrail ($n = 237$)	Frail ($n = 73$)	
Age, mean (SD)	77.7 (6.0)	76.5 (5.0)	78.6 (6.5)	80.3 (6.4)	<0.001
Gender, females/males	331/299	153/167	129/108	49/24	0.009
Years of education, mean (SD)	6.8 (5.3)	7.3 (5.6)	6.2 (4.9)	6.6 (5.5)	0.140
Satisfaction of economic situation (%)					0.05
Excellent	0.3	0.3	0.4	0.0	
Very good	2.4	2.2	2.5	2.7	
Good	28.1	29.4	27.4	24.7	
Regular	59.5	61.6	59.5	50.7	
Poor	9.5	6.6	9.7	21.9	
Comorbidity score[a], mean (SD)	1.5 (1.3)	1.4 (1.2)	1.6 (1.3)	1.6 (1.4)	0.212
MMSE score, mean (SD)	22.7 (3.5)	23.4 (3.4)	22.3 (3.4)	21.2 (3.9)	<0.001
Depressive symptoms (%)	14.4	6.9	16.8	40.4	<0.001
Disability ≥1 IADL task (%)	43.3	27.8	51.1	86.3	<0.001
Disability ≥1 ADL task (%)	29.3	17.9	33.2	67.6	<0.001
Frailty criteria (%)					
Weight loss	9.5	0.0	16.5	28.8	<0.001
Exhaustion	30.2	0.0	54.9	82.2	<0.001
Low physical activity level	12.9	0.0	16.5	57.5	<0.001
Slowness	18.9	0.0	21.1	94.5	<0.001
Weakness	16.2	0.0	17.3	83.6	<0.001

The variables are described using frequency and proportion or arithmetic mean and standard deviation (SD)

MMSE Mini-Mental State Examination, *IADL* instrumental activities of daily living, *ADL* activities of daily living

[a] The presence of seven chronic diseases was summed up in a score ranging from 0 to 7, where a higher score indicates more chronic diseases (myocardial infarction, stroke, cancer, hypertension, diabetes, dyslipidemia, and thyroid disease)

Table 2 Genotypic and allelic frequencies of the *IL-4* VNTR polymorphism in the study groups

	Study groups						
	Nonfrail	Prefrail			Frail		
	$n = 320$ (%)	$n = 237$ (%)	OR (CI)	*p*	$n = 72$ (%)	OR (CI)	*p*
Genotype							
RP1/RP1	80 (25)	62 (26)	–	–	19 (26)	–	–
RP1/RP2	144 (45)	114 (48)	1.02 (0.68–1.54)	0.92	35 (49)	1.02 (0.55–1.90)	0.94
RP2/RP2	96 (30)	61 (26)	0.82 (0.52–1.30)	0.39	18 (25)	0.79 (0.39–1.60)	0.51
Allele							
RP1	304 (47.5)	238 (50)	–	–	77 (55)	–	–
RP2	336 (52.5)	236 (50)	0.90 (0.71–1.14)	0.34	63 (45)	0.88 (0.61–1.26)	0.49
Phenotype							
IL-4^{High}	224 (70)	176 (74)	–	–	54 (65)	–	–
IL-4^{Low}	96 (30)	61 (26)	0.81 (0.55–1.18)	0.27	18 (25)	0.78 (0.43–1.40)	0.4

The values are presented as frequency in percentage (%) and genotypes and alleles number (*n*). The frequencies comparison between groups was analyzed using Chi-square test (χ^2) and Fisher's exact test when applicable

IL-4^{High} RP1/RP1 + RP1/RP2, *IL-4*^{Low} RP2/RP2

Table 3 Genotypic and allelic frequencies of the *IL-1RN* VNTR polymorphism in the study groups

	Study groups						
	Nonfrail	Prefrail			Frail		
	$n \geq 319$ (%)	$n = 237$ (%)	OR (CI)	p	$n = 72$ (%)	OR (CI)	p
Genotype							
A1/A1	275 (86)	198 (84)	–	–	57 (79)	–	–
A1/A3	4 (1.3)	6 (2.5)	2.08 (0.58–7.48)	0.26	1 (1.4)	1.21 (0.13–10.99)	0.87
A1/A4	4 (1.3)	3 (1.2)	1.04 (0.23–4.71)	0.96	0 (0)	NA	–
A2/A2	29 (9.1)	25 (10.5)	1.19 (0.68–2.11)	0.53	11 (15.3)	1.83 (0.86–3.87)	0.11
A3/A3	4 (1.3)	1 (0.4)	0.34 (0.04–3.13)	0.35	1 (1.4)	1.21 (0.13–10.99)	0.87
A4/A4	3 (0.94)	4 (1.7)	1.85 (0.41–8.37)	0.42	2 (2.8)	3.22 (0.52–19.69)	0.21
Allele							
A1	558 (87.5)	405 (85.4)	–	–	115 (80)	–	–
A2	58 (9)	50 (10.6)	1.19 (0.79–1.77)	0.4	22 (15.3)	1.84 (1.08–3.12)	0.02
A3	12 (1.9)	8 (1.7)	0.92 (0.37–2.27)	0.85	3 (2)	1.21 (0.33–4.37)	0.76
A4	10 (1.6)	11 (2.3)	1.52 (0.64–3.60)	0.35	4 (2.7)	1.94 (0.59–6.29)	0.27
Phenotype							
IL-1Ra^{Low}	290 (70)	176 (74)	–	–	61 (84.7)	–	–
IL-1Ra^{High}	29 (9.1)	25 (10.5)	1.42 (0.80–2.50)	0.22	11 (15.3)	1.80 (0.85–3.81)	0.12

The data are presented as frequency in percentage (%) and genotypes and alleles number (n). The frequencies comparison between groups was analyzed using Chi-square test (χ^2) and Fisher's exact test when applicable

NA not applicable, *IL-1Ra*^{High} A2/A2, *IL-1Ra*^{Low} non A2/A2

polymorphisms ($p > 0.05$; data not shown). Genotypic and allelic frequencies of the *IL-4* VNTR polymorphism did not show significant differences between the study groups (nonfrail, prefrail and frail subjects) ($p > 0.05$) (Table 2).

According to the different tandem repeats of the *IL-1RN* VNTR polymorphism, five types of alleles can be recognized: A1 (four repeats, 420 bp), A2 (two repeats, 240 bp), A3 (five repeats, 498 bp), A4 (three repeats, 326 bp) and A5 (six repeats, 595 bp) [28]. In our study, just four alleles (A1, A2, A3 and A4) were recognized. The genotypic and allelic frequencies of this VNTR polymorphism in study groups are shown in Table 3.

In this case, we did not find significant differences ($p > 0.05$) in the genotypic frequencies of the *IL-1RN* VNTR polymorphism between the study groups (nonfrail, prefrail and frail subjects). However, when we compared the allelic frequencies, we observed a significant difference for A2 allele between frail groups versus nonfrail group ($p = 0.02$), with an OR of 1.84 (95 % CI 1.08–3.12), indicating an association between *IL-1RN* VNTR polymorphism and frailty (Table 3).

In addition, due to the biologic relationship in inflammatory responses between both study genes, it was of our interest to investigate the gene–gene interaction. For this purpose, we analyzed the combined effect of *IL-4* and *IL-1RN* genes and their possible association with frailty syndrome, similar to a previous study conducted in type-2 diabetes mellitus [33]. Genotypes were grouped according to its functional impact on protein into high or low producer phenotypes.

In the case of *IL-4* VNTR polymorphism, it has been proved that this variant might influence the IL-4 production, with the RP1 allele (two 70 bp repeats) enhancing IL-4 expression compared with RP2 allele (three 70 bp repeats) [13, 15, 16]; thus, the RP1/RP1 or RP1/RP2 genotypes were named high producer (HP) phenotypes and RP2/RP2 genotype was named low producer (LP) phenotype.

With regard to *IL-1RN* VNTR polymorphism, it is known that elevated production of IL-1RN is an excellent marker of disease and certainly a better indicator than IL-1 itself [22] and it has been reported that A2 allele is associated with increased IL-1Ra levels, compared with the others alleles [18, 34]; therefore, the A2/A2 genotype was named HP phenotype and all other genotypes were considered LP phenotypes, resulting in four combined genotypes for both *IL-4* and *IL-1RN* genes VNTR polymorphisms (*IL-4*^{low}*–IL-1Ra*^{low}, *IL-4*^{low}*–IL-1Ra*^{high}, *IL-4*^{high}*–IL-1Ra*^{low} and *IL-4*^{high}*–IL-1Ra*^{high}).

The comparative analysis of the combined *IL-4* and *IL-1RN* genotypes in the participants according to frailty status showed that frequencies of all these combinations between prefrail and nonfrail groups lacked of significant difference ($p = 0.05$) (Table 4). However, in the

Table 4 Analysis of the combined *IL-4* and *IL-1RN* genotypes in the study groups

Groups	Combined genotypes			
	IL-4^{low}–*IL-1Ra*^{low}	*IL-4*^{low}–*IL-1Ra*^{High}	*IL-4*^{High}–*IL-1Ra*^{low}	*IL-4*^{High}–*IL-1Ra*^{High}
Nonfrail, $n = 319$ (%)	92 (28.8)	3 (0.94)	199 (62.4)	25 (7.8)
Prefrail, $n = 234$ (%)	57 (24)	4 (1.7)	155 (65.4)	21 (8.9)
OR (CI)	0.79 (0.54–1.17)	1.83 (0.41–8.26)	1.18 (0.83–1.68)	1.16 (0.63–2.12)
p	0.24	0.43	0.35	0.63
Frail, $n = 72$ (%)	13 (18)	5 (7)	48 (66.7)	6 (8.3)
OR (CI)	0.54 (0.28–1.04)	7.86 (1.83–33.69)	1.20 (0.70–2.07)	1.06 (0.42–2.71)
p	0.06	0.006*	0.49	0.89

The analysis of combined genotypes was carried out using genetic data analysis: computer program for the analysis of allelic data. The comparison data were evaluated by Chi-square test (χ^2) and Fisher's exact test when applicable

* Fisher's exact test

frequencies comparison of the combined *IL-4*^{low}–*IL-1Ra*^{high} genotype between frail and nonfrail groups, we identified a significant difference (OR 7.86, 95 % CI 1.83–33.69, $p = 0.006$) (Table 4), suggesting that the combined *IL-4*^{low}–*IL-1Ra*^{high} genotype is a marker of risk to frailty syndrome.

Finally, with respect to the socio-demographic characteristics and health status according to both *IL-4* and *IL-1RN* VNTR polymorphisms in our study groups (frail, prefrail and nonfrail), we did not find association between any of these polymorphisms and the different characteristics evaluated ($p > 0.05$; data not shown).

Discussion

Chronic inflammation is a pathophysiologic process that contributes directly to frailty and indirectly through other intermediate physiologic systems [1]. Several studies have shown a heightened inflammatory state in frail older adults, marked by high serum levels of inflammatory mediators, such as cytokines, supporting the existence of a dysregulated immune system in frail older adults [35]. In addition, among older adults, there is considerable variation in the effects of age on physiological functions, with some subjects showing an important and accelerated decline, whilst others exhibit a high resistance to this process [36, 37]. Thus, the identification of older individuals who are frail or at risk of becoming frail with appropriate subsequent evaluation and intervention constitutes a keystone of geriatric medicine and quality care for the ever-growing elderly population [1].

In this regard, although aging predisposes individuals to frailty, not all elderly adults are frail, some are able to lead full and active lives at home while others become unable to adapt to environmental challenges, suggesting that individual genetic component has a possible role in the development to frailty syndrome. Considering these facts and taking into consideration that the genetic susceptibility to frailty syndrome in the Mexican elderly adults has received little or null attention, the main interest of this study was to evaluate two important biomarkers of the inflammation process in the context of the frailty syndrome and, to our knowledge, this is the first study that analyzes the association of the *IL-4* and *IL-1RN* VNTR polymorphisms and their combined effect on frailty syndrome in Mexican community-dwelling elderly.

Our research provides evidence, for the first time, that the A2 allele of the *IL-1RN* VNTR polymorphism is significantly associated with an increased risk of frailty syndrome. However, in the case of the *IL-4* VNTR polymorphism we did not find an association of this polymorphism with susceptibility to frailty. Moreover, the genotype and allele frequencies of this genetic variant have not been reported previously; therefore, it would be necessary to perform further association studies in different populations with a large number of samples of frail elderly, to confirm this result. In addition, since just one VNTR polymorphism has been found associated with the risk of frailty, it was imperative to investigate if the combination of these particular genetic markers in the *IL-4* and *IL-1RN* genes could specifically influence the frailty susceptibility. In addition, we also tried to find out the combined effect of *IL-4* and *IL-1RN* genes on frailty syndrome and observed that *IL-4*^{low}–*IL-1Ra*^{high} genotype showed 7.86 times more risk to become frail elderly. To our awareness, there are no similar studies that have shown this association, which could allow us to compare our results.

Consequently, these findings provide preliminary evidence that supports the possible relationship between several genetic polymorphisms within genes encoding proteins

involved in the inflammatory process and the frailty phenotype in our population of Mexican elderly. In this regard, Almeida et al., conducted a cross-sectional study in Australian community-dwelling elderly to determine if polymorphisms in C-reactive protein (CRP) an inflammatory marker, were associated with frailty. They analyzed two single-nucleotide polymorphisms (SNPs) in *CRP* gene, the CRP1444C>T and CRP1846G>A, and showed that the odds of frailty were nearly 2.5 times greater among older Australian men homozygote for the minor as compared with the major allele of the CRP1846G>A polymorphism [38], also, supporting the fact that several inflammatory markers may play a role in the susceptibility to frailty syndrome and late-life physical decline.

This observation differs with a study reported by Ho et al. where they selected and analyzed a broad set of candidate gene based on their roles in the physiological systems which most likely influence frailty, skeletal, muscle and inflammation. They found that none of the SNPs within inflammatory and muscle genes were among the SNPs associated with susceptibility to frailty in their population; they only observed that genes involved in apoptotic and transcription regulation pathways were strongly associated with frailty rather than inflammation and muscle maintenance per se [8], and these results are consistent with previous gene expression studies in the skeletal muscle of a frail mouse model that showed a significant upregulation in the expression of genes related to apoptosis and a downregulation in genes related to biosynthesis and transcriptional regulation [39].

In addition, Moore et al., investigated the possible association between mitochondrial DNA (mtDNA) polymorphisms and frailty and found that three mtDNA SNPs were significantly associated with the frailty phenotype. An example is the study developed by Moore et al. which examined the possible association between mitochondrial DNA (mtDNA) polymorphisms and frailty, and found that three mtDNA SNPs were significantly associated with the frailty phenotype [40]. Likewise, two recent studies showed that SNPs in genes that influence one-carbon metabolism and vitamin B12 pathways, such as polymorphisms within *TCN2* gene, were significantly associated with the frailty syndrome [41, 42]. The divergence of the findings among the previous studies and the present study could be explained by ethnical differences of the populations studied and the environmental and socioeconomic factors to which the elderly have been exposed in each country through the life cycle.

But mainly, these results support and are consistent with phenotypic evidence that frailty is a clinical syndrome, and provides additional genetic sustenance for the clinical theory and evidence reported in the literature that establishes that frailty is a syndrome that combining multiple physiological systems, and is not driven by one single component [8]. Thus, this opens the possibility that the pathophysiology of frailty could be a two-step process in which a group of genes control susceptibility to frailty per se, while others control the clinical manifestations. Nevertheless, because the study of frailty is an ongoing challenge within current geriatric research, and due to the few studies that have evaluated the genetic risk for this syndrome, more studies in this field are needed across different cohorts to determine the specific role of these genetic markers in the frailty phenotype and to identify their high potential for providing inputs that could be used in the planning of elderly care strategies and prevention of frailty in aging through early targeted interventions.

In conclusion, our results suggest that the A2 allele of the *IL-1RN* VNTR polymorphism as well as the combined *IL-4*^{low}–*IL-1Ra*^{high} genotype are genetic markers of susceptibility to frailty in older Mexican adult. However, further studies are required to evaluate the molecular and functional basis of these associations in frailty syndrome.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest related to the publication of this manuscript.

Ethical approval The "Coyoacan Cohort" study protocol and the informed consent format were approved by the Ethical Committees of the Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" (INCMNSZ) and the Instituto Nacional de Salud Pública (INSP) of Mexico City. Likewise, the study protocol and informed written consent for genetic studies were approved by the Ethical and Research Committees of the Instituto Nacional de Geriatría of Mexico City, and the study was performed according to the ethical guidelines of the Declaration of Helsinki.

Statement of human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all the individual participants included in the study.

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