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Genetic variants in the PPARD-PPARGC1A-NRF-TFAM mitochondriogenesis pathway are neither associated with muscle characteristics nor physical performance in elderly

Variaciones genéticas en la vía de la mitocondriogénesis PPARD-PPARGC1A-NRF-TFAM no están asociadas ni con características musculares ni con rendimiento físico en personas mayores

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Abstract

We studied the influence of genetic polymorphisms involved in the PPARD-PPARGC1A-NRF-TFAM mitochondriogenesis pathway (rs6949152, rs12594956, rs2267668, rs8192678, and rs1937) on muscle phenotypes (thigh muscles' cross-sectional, maximal handgrip-strength and 30-second chair stand-test) and Barthel index in Caucasian (Spanish) community-dwelling old people (n=75, 21 men, 54 women; 71–94 years). We found no significant genetic associations with the studied phenotypes. Multiple, complex gene-environment and gene-gene interactions which are yet to be determined are likely to play a more determinant role.

Key words: ageing; mitochondria; muscle.

Resumen

Se estudió la influencia de los polimorfismos genéticos implicados en la vía de mitocondriogénesis PPARD-PPARGC1A-NRF-TFAM (rs6949152, rs12594956, rs2267668, rs8192678 y rs1937) en distintos fenotipos musculares (sección transversal muscular del muslo, fuerza máxima de prensión manual y 30 segundos de sentarse-levantarse de una silla) y en el índice de Barthel en personas mayores caucásicas (españoles) (n = 75, 21 hombres, 54 mujeres; 71 a 94 años). No se encontraron asociaciones genéticas significativas con los fenotipos estudiados. Interacciones múltiples, complejos gen-ambiente y relaciones gen-gen aún no determinadas podrían desempeñar un papel más determinante.

Palabras clave: envejecimiento; mitocondrias; músculo.

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Introduction

Western populations are ageing. As more individuals live longer, we should try to elucidate the mechanisms involved in healthy ageing and preserving functional independence in later life. With regards to this, mitochondria are one of the most important organelles for understanding the ageing process (Vina, Gomez-Cabrera, Borras, Froio, Sanchis-Gomar, Martinez-Bello, & Pallardo, 2009b). A constant renewal of these organelles is crucial for maintaining their normal function. Yet the capacity for mitochondrial biogenesis (or 'mitochondriogenesis') as well as mitochondrial function decreases with age (Fannin, Lesnefsky, Slabe, Hassan, & Hoppel, 1999; Sugiyama, Takasawa, Hayakawa, & Ozawa, 1993). Mitochondriogenesis is critical for maintaining the functional and structural integrity of post-mitotic tissues like skeletal muscle (Nair, 2005). Thus, age-associated decreases in mitochondriogenesis are responsible for many of the deleterious effects of ageing, including loss of muscle mass and function, i.e. sarcopenia (Lopez-Lluch, Irusta, Navas, & de Cabo, 2008; Shah, Scariano, Waters, Qualls, Morgan, Pickett, Gasparovic, Dokladny, Moseley & Raj, 2009). Skeletal mitochondrial function also declines with ageing owing to decreased mitochondrial DNA and oxidative damage (Short, Bigelow, Kahl, Singh, Coenen-Schimke, Raghavakaimal & Nair, 2005).

Mitochondrial synthesis is stimulated by the proliferator-activated receptor delta (PPARD)-peroxisome proliferator-activated receptor γ coactivator 1α (PPARGC1A, also termed PGC1-α)-nuclear respiratory factor (NRF)-mitochondrial transcription Factor A (TFAM) pathway. Briefly, PPARD induces promotion of PPARGC1A (Berger & Moller, 2002), which is the first stimulator of mitochondriogenesis. The NRF1 and 2 are intermediate transcription factors that stimulate the synthesis of TFAM, and the latter is the final effector activating the duplication of mitochondrial DNA molecules (Garesse & Vallejo, 2001; Kanki, Ohgaki, Gaspari, Gustafsson, Fukuoh, Sasaki, Hamasaki & Kang, 2004; Larsson, Wang, Wilhelmsson, Oldfors, Rustin, Lewandoski, Barsh & Clayton, 1998; Puigserver, Adelmant, Wu, Fan, Xu, O´Malley & Spiegelman, 1999; Puigserver & Spielgelman, 2003; Wu, Puigserver, Andersson, Zhang, Adelmant, Mootha, Troy, Cinti, Lowell, Scarpulla & Spigelman, 1999). Growing interest has focused on elucidating the factors that can affect the PPARD-PPARGC1A-NRF-TFAM pathway. This pathway is impaired by ageing, leading to increased tendency for mitochondrially-mediated apoptosis and thus eventually to sarcopenia (Hood, 2009). Genetic variants could also affect the PPARD-PPARGC1A-NRF-TFAM pathway and several important health-related phenotypes. For instance, the rs2267668 A/G polymorphism in *PPARD* and the Gly482Ser polymorphism in *PPARGC1A* are independently implicated in the modulation of aerobic fitness or insulin sensitivity (Stefan, Thamer, Staiger, Machicao, Machann, Schick, Venter, Niess, Laakso, Fritsche & Häring, 2007), and the *PPARD* A/G (rs2267668) polymorphism has also been associated with muscle mass in adults (Thamer, Machann, Stefan, Schäfer, Machicao, Staiger, Laakso, Böttcher, Claussen, Schichk, Fritsche & Haring, 2008).

The main purpose of our study was to examine the influence of several genetic polymorphisms in the *PPARD-PPARGC1A-NRF-TFAM* pathway [*NRF1* A/G (rs6949152), *NRF2* A/C (rs12594956), *PPARD* A/G (rs2267668), *PPARGC1A* Gly482Ser (rs8192678), and *TFAM* Ser12Thr (rs1937)], on phenotypes indicative of muscle mass and function in a cohort of Caucasian (Spanish) octogenarians.

Methods

Participants

The institutional research ethics committee (*Universidad Europea de Madrid*, Spain) approved the study and it was in agreement with the ethical standards in sport and exercise science research (Harriss & Atkinson, 2011). After giving their written informed consent, 75 community-dwelling healthy elderly people (21 men, 54 women; mean age: 83±5 years; range: 71–94 years) volunteered for this investigation. All were of the same Caucasian (Spanish) descent from three or more generations and were recruited from two geriatric nursing homes located in Spain. They received a comprehensive medical examination before enrolling in the study. Inclusion criteria were: age ≥70 years, able to ambulate, able to communicate and being capable and willing to provide consent. Exclusion criteria were: acute or terminal illness, myocardial infarction in the past 3 years, unstable cardiovascular disease, upper or lower extremity fracture in the past 3 years, severe dementia and presence of neuromuscular disease or drugs affecting neuromuscular function.

Phenotype assessment

*Thigh cross sectional area***.** Magnetic resonance imaging (General Electric, 2421 N Mayfair Rd, Milwaukee, WI, USA) was used to determine the thigh muscles' cross sectional area (CSA). The participants were scanned while lying supine with the knee and hip joints extended and their arms folded over the chest. An image located nearest to 50% of the femur's length, from the lower edge of the femur to the greater trochanter, was obtained for the determination of the right thigh muscles' CSA (Garatachea, Fiuza-Luces, Torres-Luque, Yvert, Santiago, Gómez-Gallego, Ruiz & Lucia, 2011). The 50% slice, or mid-thigh level, was chosen because it is commonly used to quantify thigh muscle CSA. The images were T1-weighted (echo time 8 milliseconds; repetition time 650 milliseconds), with a 40-cm^2 field of view and a matrix of 512x384 pixels (in-plane spatial resolution of 0.78x1.78 mm). Thigh CSA was normalized to body weight.

*Muscle (upper and lower body) strength***.** Maximal handgrip strength was measured using a digital dynamometer (Smedley handy dynamometer, Sportstek, Victoria, Australia), with the scores recorded to the nearest 0.1 kg. When performing the measurement, participants were instructed to maintain the standard bipedal position during the entire test with the arm at their side in complete extension. The dynamometer could not touch any part of the body except the hand being tested. Each subject performed the test with the non-dominant arm three times (with a 30-60 second rest period between the measurements) and the highest score was recorded. The grip span of the dynamometer was adjusted to the individual's hand size (Ruiz-Ruiz, Mesa, Gutierrez & Castillo, 2002).

Lower body muscle fitness was assessed with the 30-second chair stand test. This test is a valid and reliable indicator (ICC=0.92) of lower body strength in community-dwelling old adults as the ones we studied here, aged ≥60 years (Jones, Rikli, & Beam, 1999; Rikli & Jones, 1999). Participants were asked to sit on a chair with arms crossed at the wrists and held against the chest. Participants completed as many 'stand ups' as possible within 30 seconds. The score was the total number of stands executed correctly within 30 seconds and normalized to body weight.

Functional ability during ADLs (Barthel index). The *Barthel index* is an instrument widely used to measure the capacity of a person for the execution of ten basic activities in daily life, obtaining a quantitative estimation of the subject's level of independency (Collin, Wade,

Davies, & Horne, 1988; Mahoney & Barthel, 1965). The ten items include: eating, transferring from bed to chair, using the toilet, bathing/showering, personal hygiene (e.g. tooth brushing, shaving) dressing, walking, stair climbing, and bowel and bladder control. Each individual item is scored with 0 (i.e. unable to perform without complete help or fecal/urine incontinence), 5 (i.e. able to perform the activity with little help or only accidental fecal/urine incontinence) or 10 (i.e. able to perform without any help or total fecal/urine continency). The sum-score ranges from 0 (*totally dependent*) to 100 (*totally independent*). In our study, we used the Spanish, validated version of the Barthel index (Baztán, Pérez del Molino, Alarcón, San Cristóbal, Izquierdo & Manzarbeitia, 1993).

Yale Physical Activity Survey (YPAS). We recorded the participants' levels of physical activity with the Yale Physical Activity Survey (YPAS). The YPAS is an interviewer-administered questionnaire for assessing physical activity in older adults (Dipietro, Caspersen, Ostfeld, & Nadel, 1993). Time spent in each type of physical activity (physical work, exercise, and recreational activities) was multiplied by an intensity code ($kcal \cdot min^{-1}$) and then summed across all activities to create an index of weekly energy expenditure ($kcal \cdot week^{-1}$). Among physical activity questionnaires used so far, the YPAS has demonstrated adequate repeatability (Dipietro et al., 1993); total energy expenditure values obtained with this questionnaire correlate positively with accelerometer (Caltrac) activity units and negatively with body weight (Dipietro et al., 1993). We used the Spanish, validated version of the YPAS questionnaire (De Abajo, Larriba, & Marquez, 2001).

'Elderly muscle score'. We also computed an overall 'elderly muscle score' by combining the standardized values of the four aforementioned phenotypes (CSA, upper and lower body strength, Barthel index) as follows (Garatachea et al., 2011): (i) phenotype results were standardized for each gender by calculating the ratio of (value – mean) divided by standard deviation (SD); (ii) the final score was calculated as the sum of the four standardized scores.

Muscle performance index. A 'muscle performance index' was calculated as follows: 30-second stand test (number of stands)/thigh CSA (m^2) (Garatachea et al., 2011).

Genotyping

During 2011, we extracted genomic DNA from saliva samples of the participants and performed genotype analyses in the genetics laboratory of the *Universidad Europea de Madrid* (Spain). Two saliva samples were obtained from each subject before the physical activity test. Moreover, our study followed the recommendations for replicating genotype–phenotype association studies (Chanock et al., 2007).

DNA purification. Genomic DNA was extracted from peripheral blood anti-coagulated with EDTA according to standard phenol/chloroform procedures followed by alcohol precipitation.

Polymorphism identification. Allelic discrimination analysis was performed with predesigned Applied Biosystems TaqMan® SNP Genotyping Assays on demand for the five polymorphisms: *PPARD* A/G (rs2267668) (ID: C__15872729_10), *PPARGC1A* Gly482Ser (rs8192678) (ID: C___1643192_20), *NRF1* A/G (rs6949152) (ID: C__29144830_10), *NRF2* A/C (rs12594956) (ID: C__32072163_20), and *TFAM* Ser12Thr (rs1937) (ID: C___8975662_10).

PCR amplification was performed using a StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA), with a denaturation stage at 95°C for 10 min, 50 cycles of

denaturation at 92°C for 15 sec, annealing/extension at 60°C for 1 min, and a final extension stage of 30 sec at 60°C.

Data analysis

Hardy–Weinberg equilibrium (HWE) was tested using the χ^2 test. In order to analyze the *individual* effects of the five polymorphisms, we analyzed the differences in the studied phenotypes among genotypes of the polymorphisms by one-way analysis of covariance (ANCOVA), where the polymorphism was entered as a fixed factor, the phenotype was entered as a dependent variable, and age, sex and physical activity levels (weekly energy expenditure) were entered as covariates. Since the frequency of the *PPARD* GG genotype was only of n=2 and that of the *NRF1* GG was only of n=1, we grouped *PPARD* GG and AG (total n=24) and *NRF1* AG and AA (total n=26) respectively. All the ANCOVA tests (including the combined (polygenic) approach that is described below) were performed with men and women together, and sex and physical activity levels were included as additional covariates to the model. For the *TFAM* Ser12Thr polymorphism, all subjects carriers of the variant allele were women; for this reason, the sex**TFAM* interaction was not performed. Analyses were corrected for multiple comparisons using the Bonferroni method, in which the threshold *P*-value is obtained by dividing 0.05 by the number of comparisons, i.e. n=6, corresponding to the 6 phenotypes we studied (thigh CSA, handgrip, 30-second chair stand, Barthel index, elderly muscle score and muscle performance index). Thus, threshold *P*-value was 0.008.

On the other hand, we compared the *NRF1* A/G*, NRF2* A/C, *PPARD* A/G*, PPARGC1A* Gly482Ser, and *TFAM* Ser12Thr genotypes between those with low 'elderly muscle' scores ($<25^{th}$ sex-specific percentile) and those with high scores ($\geq 25^{th}$ sex-specific percentile) with the χ^2 test. Finally, we used logistic regression to calculate the odds ratio for having 'accelerated' sarcopenia (i.e. 'elderly muscle score' $<25^{th}$ sex-specific percentile) after adjusting for age, sex and physical activity levels. [The odds ratio, (OR) is the ratio of the odds of an event (e.g. 'sarcopenia') occurring in one group (e.g. individuals with one genotype) to the odds of it occurring in another group (e.g. individuals with another genotype)].

All the analyses were performed with the PASW/SPSS Statistics 18.0 (SPSS Inc, Chicago, IL), and the level of significance was set at $\alpha \leq 0.05$.

Results

There were no failures in sample collection, DNA acquisition or genotyping procedures, except for *PPARGC1A* Gly482Ser genotyping in one participant, and *NRF2* A/C genotyping in other participant, due in both cases to an insufficient amount of DNA gathered from saliva (fig. 1). Genotype frequencies were in HWE for all the polymorphisms we studied (Table 1).

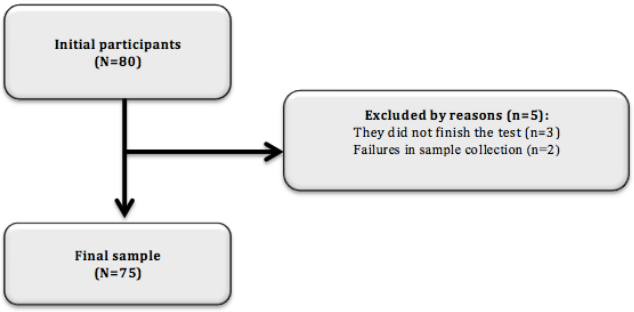

Figure 1. Sample size description.

Weekly energy expenditure in physical activities averaged 4,010 ± 3,952 kcal·week^{-1} in the whole cohort.

Table 1. Studied polymorphisms and 'genotypes scores'

Symbol (2='optimal', 0='worst')	**Gene**	**Polymorphism**	**Genotypes**
NRF2	Nuclear respiratory factor-2	A/C (rs12594956)	0=CC, 1=AC, 2=AA
PPARD	Peroxisome proliferator-activated receptor delta	A/G (rs2267668)	0=GG, 1=AG, 2=AA
PPARGC1A	Peroxisome proliferator-activated receptor-γ coactivator 1-α	Gly(G)482Ser(S) (rs8192678)	0=SS, 1=GS, 2=GG
TFAM	Transcription factor A mitochondrial	Ser(S)12Thr(T) (rs1937)	0=SS, 1=ST, 2=TT

Therefore, The mean phenotype values across the different genotypes are shown in Table 2. We did not observe any interaction effect of sex*polymorphism (P>0.05) on the phenotypes. We found no significant differences in muscle phenotypes across genotypes (all P>0.05), except for a significant gen*sex interaction in thigh CSA for *PPARGC1A* Gly482Ser (P=0.030), and 30-second chair stand test for *NRF2* A/C (P=0.036). Yet the two aforementioned P-values were above the 0.008 threshold established for correcting multiple comparisons with the Bonferroni method.

Table 2. Genotype frequencies for the different polymorphisms

Polymorphism				**HWE (*P*-value)**
NRF1 A/G (rs6949152)	AA=49	AG=25	GG=1	0.263
NRF2 A/C (rs12594956)	AA=38	AC=28	CC=8	0.416
PPARD A/G (rs2267668)	AA=51	AG=22	GG=2	0.838
PPARGC1A Gly(G)482Ser(S) (rs8192678)	GG=26	GS=36	SS=12	0.937
TFAM Ser(S)12Thr(T) (rs1937)	SS=66	ST=9	TT=0	0.588

Abbreviations: HWE, Hardy-Weinberg equilibrium. See text for gene abbreviations.

We found no significant differences in genotype frequency distributions between those participants with a lower or higher 'elderly muscle score' respectively (P>0.05) (Figure 1). On the other hand, logistic regression analysis showed that participants with one genotype had no increased OR (or 'chance') of having accelerated sarcopenia when compared to each other genotype (Table 3).

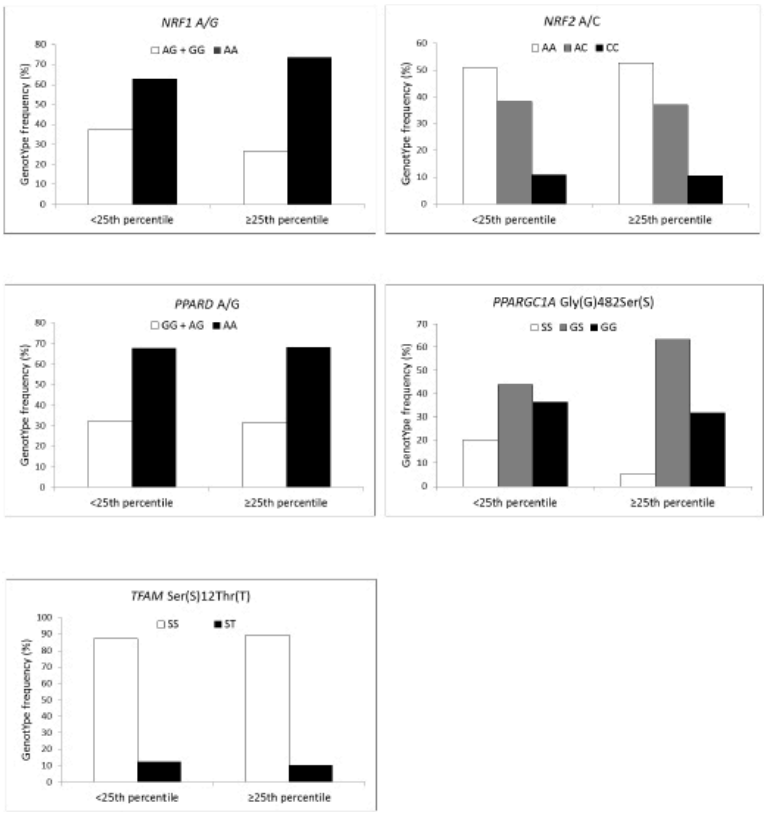

Figure 1. Genotype distributions of each studied genotypes by 'elderly muscle scores' (see text for explanations on score calculation). No differences existed across genotypes for the different polymorphisms.

(*NRF1* A/G: $\chi^2 = 0.320$, $P = 0.852$; *NRF2* A/C: $\chi^2 = 0.572$, $P = 0.751$; *PPARD* A/G: $\chi^2 = 0.608$, $P = 0.738$; *PPARGC1A* Gly482Ser: $\chi^2 = 2.829$, $P = 0.243$; *TFAM* Ser12Thr: $\chi^2 = 0.001$, $P = 0.973$).

Test-retest reliability of our handgrip measurements was high [intra-class correlation coefficient (ICC) of 0.936 (95% confidence intervals (CI): 0.887-0.964; P<0.001)].

Table 3: Mean ± SD estimates of muscle phenotypes by genotypes of the studied polymorphisms.

***NRF1* A/G (rs6949152)**	**AA (n=49)**	**AG + GG (n=26)**		***P***
Thigh CSA (mm^2)	9,859.1 ± 2,279.4	10,557.7 ± 2,413.9		0.133
Handgrip (kg)	22.3 ± 8.0	21.5 ± 8.8		0.333
30-second chair stand test (Nº)	10.1 ± 4.6	9.9 ± 4.5		0.396
Barthel index	92.4 ± 11.4	95.6 ± 7.4		0.508
'Elderly muscle score'	-0.7 ± 4.0	-0.4 ± 3.8		0.209
'Muscle performance index'	0.1 ± 0.0	0.1 ± 0.0		0.686
***NRF2* A/C (rs12594956)**	**AA (n=38)**	**AC (n=28)**	**CC (n=8)**	***P***
Thigh CSA (mm^2)	10268.8 ± 1607.3	9906.4 ± 2206.3	10225.6 ± 2589.9	0.546
Handgrip (kg)	24.6 ± 8.1	21.0 ± 7.4	22.0 ± 8.7	0.824
30-second chair stand test (Nº)	10.9 ± 3.6	9.5 ± 3.6	10.1 ± 5.3	0.036*
Barthel index	92.5 ± 7.6	95.7 ± 9.0	91.8 ± 11.7	0.294
'Elderly muscle score'	-1.0 ± 3.9	0.0 ± 3.5	-0.9 ± 4.3	0.137
'Muscle performance index'	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.053
***PPARD* A/G (rs2267668)**	**AA (n=51)**	**GG + AG (n=24)**		***P***
Thigh CSA (mm^2)	9,841.4 ± 2,381.4	10,687.3 ± 2,171.0		0.954
Handgrip (kg)	20.9 ± 8.4	24.5 ± 7.5		0.140
30-second chair stand test (Nº)	9.7 ± 4.4	10.8 ± 4.9		0.430
Barthel index	93.8 ± 9.8	92.9 ± 11.5		0.337
'Elderly muscle score'	-1.0 ± 3.9	0.2 ± 3.8		0.931
'Muscle performance index'	0.1 ± 0.0	0.1 ± 0.0		0.362
***PPARGC1A* Gly(G)482Ser(S) (rs8192678)**	**GG (n=26)**	**GS (n=36)**	**SS (n=12)**	***P***
Thigh CSA (mm^2)	9,997.1 ± 2,583.2	10,039.4 ± 2,025,4	10,408.7 ± 2,891.2	0.030*
Handgrip (kg)	22.6 ± 8.8	20.9 ± 7.9	23.5 ± 8.4	0.870
30-second chair stand test (Nº)	9.8 ± 4.1	9.4 ± 4.7	12.5 ± 4.9	0.300
Barthel index	96.0 ± 8.2	91.3 ± 12.0	94.1 ± 8.0	0.363

'Elderly muscle score'	-0.6 ± 3.5	-0.9 ± 4.2	-0.1 ± 3.9	0.144
'Muscle performance index'	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.944
***TFAM* Ser(S)12Thr(T) (rs1937)**	**SS (n=66)**	**ST (n=9)**	**TT (n=0)**	***P***
Thigh CSA (mm^2)	10,068.6 ± 2,440.3	10,387.6 ± 1,451.6		
Handgrip (kg)	22.6 ± 8.6	18.0 ± 2.6		
30-second chair stand test (Nº)	10.4 ± 4.7	7.5 ± 2.1		
Barthel index	93.3 ± 10.4	94.4 ± 9.8		
'Elderly muscle score'	-0.7 ± 4.1	0.1 ± 2.5		
'Muscle performance index'	0.1 ± 0.0	0.1 ± 0.0		

*See text for gene abbreviations and for explanation of the 'elderly muscle score' and 'muscle performance index'. Abbreviations: CSA, cross-sectional area. Symbol: * statistical significance was not reached after correction for multiple comparisons (threshold P-value=0.008).*

*For the TFAM Ser(S)12Thr(T) polymorphism, all subjects carriers of the variant allele were women; for this reason, the sex*TFAM interaction was not performed.*

PPARD-PPARGC1A-NRF-TFAM pathway [*NRF1* A/G (rs6949152), *NRF2* A/C (rs12594956), *PPARD* A/G (rs2267668), *PPARGC1A* Gly482Ser (rs8192678), and *TFAM* Ser12Thr (rs1937)].

Discussion

This is a novel study on the potential influence of mitochondriogenesis-related genes on muscle phenotypes in Caucasian old people, suggesting that genetic polymorphisms involved in the PPARD-PPARGC1A-NRF-TFAM pathway do not have a major individual or combined effect on such phenotypes in this population.

Loss of mitochondriogenesis is critical in maintaining the functional and structural integrity of post-mitotic tissues like skeletal muscle (Fitzmaurice, 2002; Nair, 2005; Vieira & Corrente, 2011). Thus, to identify genotypes related to mitochondriogenesis (involving the PPARD-PPARGC1A-NRF-TFAM pathway) is of interest from a broader health perspective in aged people because these genotypes theoretically could be associated with muscle capacity and frailty in the elderly. Indeed, mitochondriogenesis is critical for the normal function of cells and the PPARD-PPARGC1A-NRF-TFAM pathway is impaired by chronic physical inactivity (Vina, Gomez-Cabrera, Borras, Froio, Sanchis-Gomar, Martinez-Bello & Pallardo, 2009a) and ageing (Hood, 2009). Reduced mitochondriogenesis can result in reduced muscle aerobic capacity (which is in itself an important health indicator), increased tendency for mitochondrially-mediated apoptosis, and, ultimately, accelerated sarcopenia (Hood, 2009). Further, research on animal models shows that impaired mitochondrial function (due to decreased amounts of transcription factors required for mitochondriogenesis) might be the

link explaining the association between low fitness levels and increased cardiovascular/metabolic disease risk (Wisloff, Ellingsen, Haram, Swoap, Al-Share, Fernström, Rezaei, Lee, Koch & Britton, 2005).

To the best of our knowledge, no data are available in the literature concerning the association of genetic polymorphisms in the PPARD-PPARGC1A-NRF-TFAM pathway with muscle phenotypes in the elderly, despite the rationale for studying such association from a broad health perspective. Indeed, some genetic polymorphisms in this pathway are associated with important health-related phenotypes, e.g. the A/G (rs2267668) polymorphism in *PPARD* and the Gly482Ser polymorphism in *PPARGC1A* were independently implicated in the modulation of aerobic fitness and insulin sensitivity in prediabetic (or with a family history of type 2 diabetes) adults (Stefan et al., 2007). Previous research on adults with increased risk for type 2 diabetes (mean age: 47 years) has shown that carriers of the minor allele in the *PPARD* A/G (rs2267668) polymorphism had less relative leg muscle volume with their peers who were homozygous for the major allele (Thamer et al., 2008). Differences between our cohort (showing no association for the rs2267668 polymorphism) and that in the study by Thamer et al. (Thamer et al., 2008), particularly with respect to age, make comparisons difficult.

The results of our study could be overall valid, as all the following criteria were met (Attia, Ioannidis, Thakkinstian, McEvoy, Scott, Minelli, Thompson, Infante-Rivard & Guyatt, 2009): phenotypes were accurately assessed, participants were ethnically matched, genetic assessment was accurate and the HWE was not violated, and we adjusted our statistical analyses for multiple comparisons. However, the low sample size of our cohort does considerably limit the 'external validity' (and therefore generalizability) of our results. In our opinion this limitation could be partly overcome by the fact that several valid muscle-related phenotypes were consistently and reliably assessed by the same researchers. This eliminates a possible confounder that exists in most multi-centre studies with larger samples, i.e. phenotype data are assessed by different investigators and analyzed together, yielding a considerable source of variability. The lack of data from a 'replication' cohort of a different ethnic background is also to be kept in mind. Thus, more research is needed using our model on larger groups of different ethnic backgrounds. On the other hand, we believe there are strengths in our design. We measured a muscle fitness test of practical applicability in the daily life of old people as is the 30-second chair sit-stand test (e.g. vs. less 'applicable' tests as one repetition maximum strength tests) (Gotshalk, Volek, Staron, Denegar, Hagerman & Kraemer, 2002) and we adjusted our analyses by a main confounder in the potential association between genes and muscle and exercise-related phenotypes, that is, physical activity levels. Finally, though there exist between-sex differences in the muscle strength and tendon characteristics of elderly people (Flueck, Eyeang-Békalé, Héraud, Girard, Gimpl, Seynnes, Rittweger, Niebauer, Mueller & Narici, 2011), the potential confounding effect of sex was well controlled for in our analyses.

Western societies are ageing and thus, sarcopenia is becoming a growing health problem. To identify those lifestyle and genetic factors that influence muscle fitness and functional capacity at the end of the human lifespan, and that could be associated with more severe sarcopenia is of clinical and public health interest. Though they are candidates to modulate exercise and health-related phenotypes in younger adults, our data suggest that polymorphisms associated with mitochondrial biogenesis do not exert a major influence in the muscle phenotypes of old people.

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