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# Prevalence and association of single nucleotide polymorphisms with sarcopenia in older women depends on definition

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The prevalence of sarcopenia depends on the definition used. There are, however, consistent sarcopenic characteristics, including a low muscle mass and muscle strength. Few studies have investigated the relationship between sarcopenia and genotype. A cross-sectional study was conducted with 307 community-dwelling  $\geq 60$ -year-old women in South Cheshire, UK. Handgrip strength was assessed with a handgrip dynamometer and skeletal muscle mass was estimated using bioelectrical impedance. DNA was extracted from saliva ( $\sim 38\%$ ) or blood ( $\sim 62\%$ ) and 24 single-nucleotide polymorphisms (SNPs) were genotyped. Three established sarcopenia definitions - %Skeletal Muscle Mass (%SMM), Skeletal Muscle Mass Index (SMI) and European Working Group on Sarcopenia in Older People (EWGSOP) - were used to assess sarcopenia prevalence. Binary logistic regression with age as covariate was used to identify SNPs associated with sarcopenia. The prevalence of sarcopenia was: %SMM 14.7%, SMI 60.6% and EWGSOP 1.3%. Four SNPs were associated with the %SMM and SMI definitions of sarcopenia; *FTO* rs9939609, *ESR1* rs4870044, *NOS3* rs1799983 and *TRHR* rs7832552. The first three were associated with the %SMM definition, and *TRHR* rs7832552 with the SMI definition, but none were common to both sarcopenia definitions. The gene variants associated with sarcopenia may help proper counselling and interventions to prevent individuals from developing sarcopenia.

Sarcopenia is defined as an ageing-related loss of both muscle mass and strength below a threshold level<sup>1</sup>. It is an important predictor of adverse outcomes such as limited mobility, increased risk of falls, decreased quality of life (QoL), hospitalization and mortality, and contributes to tens of millions of pounds of health care costs in the UK<sup>1-3</sup>. Although muscle weakness and skeletal muscle atrophy are overt characteristics of this geriatric syndrome, there is ongoing debate on the operational definition, screening and diagnosis, and optimal management and treatment of the condition<sup>1,4-6</sup>. The considerable heterogeneity in the reported prevalence of sarcopenia is largely attributable to the different definitions or cut-offs used<sup>7-9</sup>.

The fact that some elderly do not show sarcopenia, whilst others of the same age do<sup>10,11</sup>, suggests that some individuals are more susceptible to sarcopenia than others. The different susceptibility is likely due to a combination of factors including physical activity, diet, sedentary behaviour and genetics<sup>12-15</sup>. Several studies have reported an association of single nucleotide polymorphisms (SNPs) with lean mass, muscle volume and muscle strength<sup>16-18</sup>. It is thus possible that individuals carrying favourable gene variants are less susceptible to sarcopenia and hence can maintain independence until later life. To date, five studies have investigated the association of SNPs with sarcopenia; limited to *VDR*, *IL6*, *ACTN3* and *MSTN* polymorphisms<sup>19-23</sup>. The studies identified an association of *ACTN3* and *VDR* gene variants with sarcopenia, but did not find any association with *IL6* and *MSTN* variants. Unlike the previous studies that used low appendicular skeletal muscle mass as the cut-off for sarcopenia<sup>19,20,22</sup>, the later studies used both low muscle mass and muscle function to define sarcopenia<sup>21,23</sup>.

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Sarcopenia definitions (in columns)   Variables (in rows)	General characteristics (n = 307)	SMI		%SMM		EWGSOP	
		S	NS	S	NS	S	NS
		186 (60.6%)		45 (14.7%)		4 (1.3%)	
Age (years)	70.7 ± 5.7	71.0 ± 5.3	70.3 ± 6.3	71.6 ± 5.6	70.6 ± 5.7	76.8 ± 7.0*	70.6 ± 5.7
Body Mass (kg)	66.3 ± 11.3	63.4 ± 9.3**	70.9 ± 12.5	77.4 ± 13.1**	64.4 ± 9.8	68.5 ± 10.9	66.3 ± 11.3
BMI (kg/m <sup>2</sup> )	25.9 ± 4.2	24.7 ± 3.3**	27.8 ± 4.7	30.2 ± 5.5**	25.2 ± 3.4	28.2 ± 2.9	25.9 ± 4.2
HGS (kg)	29.9 ± 5.0	29.1 ± 4.4**	31.1 ± 5.6	28.4 ± 4.9*	30.2 ± 5.0	17.8 ± 2.2**	30.0 ± 4.9
SMI (kg/m <sup>2</sup> )	6.56 ± 0.81	6.05 ± 0.51**	7.33 ± 0.53	6.04 ± 0.92**	6.64 ± 0.75	6.29 ± 0.18	6.56 ± 0.81

**Table 1.** Population and sarcopenia group characteristics with different sarcopenia definition. \*And \*\* denote difference from non-sarcopenia group at  $p < 0.05$  and  $p < 0.001$  respectively. BMI, Body Mass Index, HGS, Hand Grip Strength, SMI, Skeletal Muscle Mass Index, S- Sarcopenia group, NS- Non sarcopenia group, n (%), number (percentage) of sarcopenic individuals.

The genetic (as opposed to environmental) component of inter-individual differences in muscle size/strength are substantial, both in younger<sup>24,25</sup> and older adults<sup>26</sup>. To date however, research in older adults has attempted to identify the precise genetic variations responsible for sarcopenia through four candidate SNPs, and none has compared different sarcopenia definitions in that regard. It would be advantageous to do so, with desirable outcomes including a greater understanding of the differences observed and the possibility of targeting interventions at those most at risk of sarcopenia. Considering the possible association of gene variants with sarcopenia (although the results may vary with different sarcopenia definitions) and heterogeneity in sarcopenia prevalence, the objectives of the present study were to assess 1) the prevalence of sarcopenia in a population of older women according to three different definitions of sarcopenia and 2) the association between sarcopenia and more SNPs than studied previously, for each definition of sarcopenia. The three chosen definitions were: skeletal muscle mass (SMM)/body mass (%SMM) (sarcopenia defined to individuals with %SMM < 22.1%)<sup>27</sup>, SMM/height<sup>2</sup> (SMI) (sarcopenia defined to individuals with SMI ≤ 6.76 kg/m<sup>2</sup>)<sup>28</sup> and SMI and Handgrip strength (HGS) cut-offs as suggested by European Working Group on Sarcopenia in Older People (EWGSOP) (sarcopenia defined to individuals with SMI < 6.76 kg/m<sup>2</sup> and HGS < 20 kg)<sup>1</sup>.

## Results

**Prevalence of sarcopenia with different sarcopenia definitions.** The prevalence of sarcopenia according to each definition was: %SMM 14.7%, SMI 60.6% and EWGSOP 1.3%. The general characteristics of the participants with each definition are presented in Table 1. Individuals identified as sarcopenic with EWGSOP were also sarcopenic by the SMI definition, while there was a discrepancy in individuals identified as sarcopenic between the SMI and %SMM definitions: 34 elderly women were sarcopenic using both the %SMM and SMI definitions, but 11 were sarcopenic using only the %SMM definition and 152 were sarcopenic using only the SMI definition. Similarly, two individuals were common to both %SMM and EWGSOP definitions, but 2 were with the EWGSOP only and 43 using only the %SMM definition.

**Associations of SNPs with sarcopenia according to the different definitions.** All genotypes were in Hardy Weinberg equilibrium ( $p > 0.05$ ) (presented in Supplementary Table S1). The distribution of genotypes varies with the sarcopenia definitions (presented for *FTO* rs9939609 in Fig. 1).

Based on the %SMM definition, the SNPs *FTO* rs9939609, *ESR1* rs4870044 and *NOS3* rs1799983 were associated with sarcopenia (presented in Table 2). Binary logistic regression, using age as a covariate, indicated that *FTO* rs9939609 AA homozygotes had 3.04 times higher risk of being sarcopenic than T-allele carriers (OR = 3.037, 95% confidence interval [CI] = 1.439–6.411,  $p = 0.004$ ). *ESR1* rs4870044 T-allele carriers had a 2.54 times greater risk of being sarcopenic than CC homozygotes (OR = 2.543, 95% CI = 1.273–5.079,  $p = 0.008$ ). Similarly, *NOS3* rs1799983 GG homozygotes had 2.26 times greater risk of being sarcopenic than T-allele carriers (OR = 2.257, 95% CI = 1.165–4.373,  $p = 0.016$ ).

Based on the SMI definition, elderly females with the *TRHR* rs7832552 C-allele carriers had 2.6-fold higher risk of being sarcopenic when compared with TT homozygotes (OR = 2.568, 95% CI = 1.268–5.203,  $p = 0.009$ ).

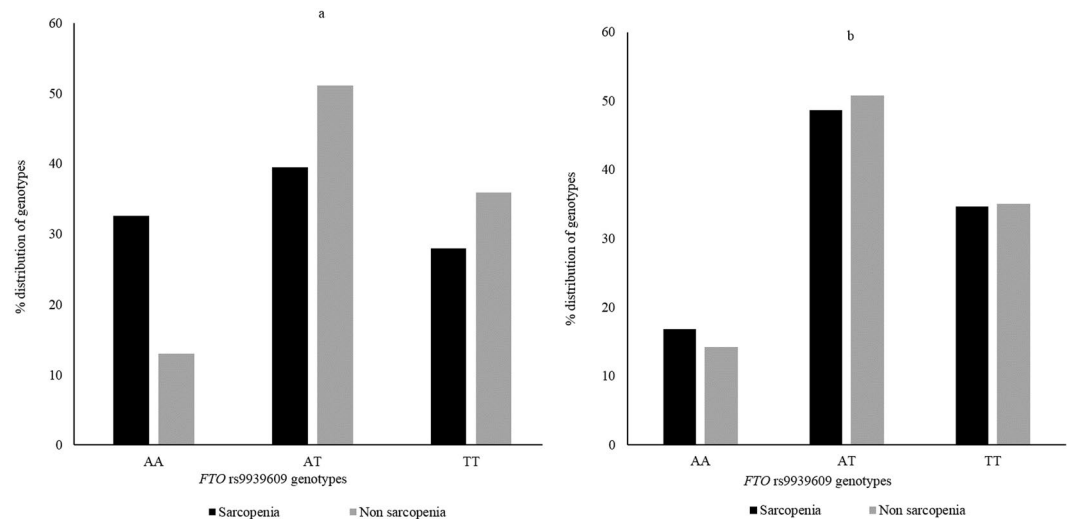
Using the EWGSOP definition, only four participants were defined as sarcopenic, therefore comparisons of genetic characteristics between sarcopenic and non-sarcopenic groups could not be performed.

None of the other 20 SNPs were associated with sarcopenia using any definition.

## Discussion

The current study identified that different definitions of sarcopenia result in a widely different prevalence of sarcopenia, ranging from 1.3% to 60.6% in the present population of women aged >60 years. Three SNPs were associated with sarcopenia based on the %SMM definition, and one SNP when using the SMI definition.

The heterogeneity of the prevalence of sarcopenia (1.3% to 60.6%), depending on sarcopenia definition used, is consistent with a previous study<sup>8</sup>. This confirms that the criteria, definition and threshold has a dramatic impact on the observed prevalence of sarcopenia, even in the same study population. Defining sarcopenia using %SMM resulted in a prevalence of 14.7% in the present elderly female population. This prevalence falls within the range in previous studies using the same approach. For example, in the large population NHANES III study 10% of US women above 60 years<sup>27</sup> and 23.6% of elderly French women<sup>29</sup> were sarcopenic according to this definition.



**Figure 1.** Distribution of *FTO* rs9939609 genotypes between sarcopenia and non-sarcopenia groups with sarcopenia definitions (a) %SMM and (b) SMI. Using %SMM, AA homozygotes had over three times higher risk of being sarcopenic than T-allele carriers (OR = 3.04,  $p = 0.004$ ).

Sarcopenia definitions	Variables	B	S.E (β)	Wald's $\chi^2$	p	OR	95% CI	Risk genotypes
SMI	Age	-0.260	0.021	1.463	0.226	0.975	0.935–1.016	CC+CT
	<i>TRHR</i> rs7832552	0.943	0.360	6.858	0.009*	2.568	1.268–5.203	
	PASE	<0.001	0.002	0.005	0.944	1.000	0.996–1.005	
%SMM	Age	-0.032	0.029	1.268	0.260	0.968	0.915–1.024	AA
	<i>FTO</i> rs9939609	1.111	0.381	8.492	0.004*	3.037	1.439–6.411	
	PASE	0.008	0.004	3.735	0.053	1.008	1.000–1.015	
	Age	-0.031	0.028	1.222	0.269	0.970	0.919–1.024	GG
	<i>NOS3</i> rs1799983	0.814	0.337	5.820	0.016*	2.257	1.165–4.373	
	PASE	0.009	0.004	4.814	0.028	1.009	1.001–1.016	
	Age	-0.031	0.028	1.308	0.253	0.969	0.918–1.023	TT+CT
	<i>ESR1</i> rs4870044	0.933	0.353	6.988	0.008*	2.543	1.273–5.079	
	PASE	0.009	0.004	4.787	0.029*	1.009	1.001–1.016	

**Table 2.** Binary logistic regression model of single nucleotide polymorphisms with age and PASE on sarcopenia. \*Denotes association of SNPs and sarcopenia in a model.

The SMI definition of sarcopenia has previously resulted in prevalence ranging from 2.8%–42.0%<sup>29–31</sup>. At the extremes, this lower prevalence in sarcopenia is due to threshold levels being set arguably too conservatively (SMI 6.2 kg/m<sup>2</sup> previously)<sup>29</sup>, compared to 6.76 kg/m<sup>2</sup> (SMI) in the present study definition consistent with previous work<sup>28</sup>. The prevalence of sarcopenia (1.3%) in elderly females in the present study using the EWGSOP definition is consistent with studies on different populations - for instance, 2.5% in Taiwanese<sup>32</sup>, 4.5% in German<sup>33</sup> and 6.8% in Japanese<sup>34</sup> women. It should be noted that higher prevalences have been reported, ranging between 22–48% in some of the studies conducted across Europe, Asia and South America<sup>35–39</sup>, but these higher prevalences tend to be in populations incorporating the oldest old (>70 years) or those not living independently and/or their own young reference group (rather than cut offs).

Within this study, elderly women identified as sarcopenic using the EWGSOP definition were also sarcopenic using the SMI definition. The SMI definition used  $\leq 6.76$  kg/m<sup>2</sup> as the cut-off for low muscle mass in defining sarcopenia, while EWGSOP identified the sarcopenic with the same cut-off for low muscle mass and additionally <20 kg handgrip strength that evidently made no material difference in our cohort. However, the discrepancy in those identified as sarcopenic between those two definitions and the %SMM definition could be because the %SMM definition uses only values of mass while the other two both adjust values of mass according to height. The difference in the prevalence of sarcopenia by considering height and body mass as normalizing factors has been observed in a previous study<sup>40</sup>. These observations clearly indicate that a consensus is needed concerning the definition of sarcopenia.

The present study identified *FTO* rs9939609 AA homozygotes to be at over 3-fold higher risk for sarcopenia compared to T-allele carriers. This genotype has been previously associated as a risk genotype for obesity-related indices such as increased BMI and fat mass<sup>41–43</sup>, and clinical conditions such as type 2 diabetes<sup>44</sup>

and tuberculosis<sup>45</sup>. The importance of *FTO* during skeletal muscle development and differentiation was reflected by the impaired skeletal muscle development in *FTO*-deficient mice, due to myogenic suppression<sup>46</sup>. There was an association between *FTO* rs9939609 AA and lean body mass; however, that association disappeared when controlling for fat mass<sup>41,47</sup>. Recent studies have reported over-representation of *FTO* rs9939609 AA genotypes in heavier athletes<sup>48,49</sup>, however, none has investigated how the body mass and muscle mass are affected by this genotype in those athletic populations. We previously demonstrated that the T-allele is associated with higher appendicular and arm lean mass in untrained young men<sup>49</sup>. It therefore appears that *FTO* AA-genotype individuals have lower skeletal muscle mass and are at higher risk of sarcopenia.

The current study is the first to identify the *NOS3* rs1799983 GG genotype as a risk factor for sarcopenia, with over 2-fold higher risk compared to T-allele carriers, for sarcopenia in the elderly. This finding is consistent with previous studies that have reported the T allele as more frequent in power oriented athletes<sup>50</sup> and female short distance swimmers<sup>51</sup> and association of TT homozygote with absolute gain in dynamic strength following resistance training in women<sup>52</sup>. Biologically, the *NOS3* gene encodes the enzyme endothelial nitric oxide synthase (eNOS) that catalyses the synthesis of nitric oxide (NO). NO has been identified as a determinant of individual variations in health and exercise related phenotypes<sup>53</sup>, mitochondrial energy production<sup>54</sup> and muscle hypertrophy<sup>55</sup>. The *NOS3* rs1799983 T allele has been associated with higher NOS activity compared to the G-allele<sup>56</sup>. The association of the *NOS3* rs1799983 T allele with a higher NOS activity and the beneficial role of NO in skeletal muscle, particularly muscle hypertrophy and energy production, provide a potential basis for the present observation that elderly women who are GG homozygotes are at higher risk of being sarcopenic.

The current study also identified the *ESR1* rs4870044 T allele as a risk factor, with 2.5-fold higher risk of sarcopenia in T-allele carriers compared to CC homozygotes. Biologically, *ESR1* encodes the oestrogen receptor protein that interacts with oestrogen and plays an important role in growth of bone and maintenance of bone mass. *ESR1* is also expressed in human skeletal muscle<sup>57</sup>. However, the role of oestrogen in skeletal muscle is still to be elucidated. The *ESR1* rs4870044 T allele has been associated previously with low bone mineral density (BMD)<sup>58,59</sup> and a higher risk of hip fracture<sup>60</sup>. Since ageing results in the deterioration of both bone and muscle, and bone geometry is partly determined by muscle mass/strength<sup>61,62</sup>, *ESR1* might contribute to both muscle and bone phenotypes<sup>63</sup>. Although previous studies did not find an association between rs4870044 and muscle strength<sup>64–66</sup>, we suggest that the discrepancy between those studies and our study might be attributable to us defining sarcopenia by the %SMM while the other studies assessed the relationship with muscle strength. Whatever the cause of the discrepancy, the higher risk of sarcopenia in T-allele carriers may be related to the physiological activities of oestrogen, mediated by oestrogen receptors<sup>67,68</sup> where lower levels of oestrogen have previously been associated with low bone mass and bone mineral density<sup>69,70</sup>, as well as low muscle mass<sup>71</sup> and muscle strength<sup>71,72</sup>.

In the current study, *TRHR* rs7832552 C-allele carriers were at over 2-fold higher risk compared to TT homozygotes for sarcopenia defined according to the SMI. This aligns with studies associating the T-allele with increased muscle mass. For example, a genome-wide association study identified individuals homozygous for the *TRHR* rs7832552 T-allele to have on average 2.5 kg more lean body mass than heterozygotes and C-allele homozygotes<sup>73</sup>. Similarly, this polymorphism has been associated with sprint/power performance<sup>74</sup>. A greater sarcopenic risk for C-allele carriers in the present elderly might be explained by lower *TRHR* expression<sup>75</sup> and hence impaired action of thyroid hormone that is considered important to preserve muscle strength<sup>76</sup>.

The prevalence and associations of sarcopenia and SNPs were studied with three different sarcopenia definitions. One limitation is that the current study was limited to older women only, but then particularly women suffer from (the consequence of) sarcopenia. Another limitation is that SMM was estimated using BIA instead of the more accurate MRI or DEXA. Although many studies have shown a good agreement (e.g. standard error of estimate 2.7 kg<sup>77</sup>) between BIA and MRI/DEXA<sup>77–79</sup>, BIA might overestimate muscle mass<sup>80,81</sup>. Therefore, future studies that adopt a more rigorous approach to assessing muscle mass (MRI or DXA) may observe stronger SNP associations than those reported in the present study. As diet<sup>82</sup> also plays an important role in the maintenance of muscle mass/strength, we also encourage future work that incorporates diet and those additional possible confounders to understand the sarcopenia mechanism in depth.

Within our study, only four of the twenty-four investigated SNPs were associated with sarcopenia. Skeletal muscle mass and strength are highly polygenic in nature<sup>83</sup>, hence the influence of any single SNP on the risk of sarcopenia is probably limited. A polygenic approach combining multiple risk alleles for sarcopenia will ultimately capture a substantial portion of the genetic risk and any future practical tool will use that approach. However, many more individual SNPs first need to be identified before a polygenic approach becomes a worthwhile development. It should also be noted that the sarcopenia definitions identified different SNPs to be associated with sarcopenia. This is probably because different individuals were identified as sarcopenic by the different definitions and demonstrates how inconsistency in the definition of sarcopenia in the literature will have consequences for the ability to identify causative factors. Given the perpetual decline of muscle mass and strength in the elderly, although individuals in our study identified as sarcopenic using the SMI and %SMM definitions (in fact they were independent and could perform daily activities without any limitations), they are probably at a higher risk of developing sarcopenia and doing so earlier than individuals not identified as sarcopenic using any definition. As a genetic association was found, SNP-related risk seems pertinent even in those elderly who are currently healthy. Similarly, the tested SNPs were not selected randomly, but due to their previous association with skeletal muscle phenotypes or similar phenotypes, so we believe the results observed are informative regarding sarcopenia-related phenotypes.

The prevalence of sarcopenia is to a large extent affected by the definition used, and ranged in our population of older women from 1.3 to 60.6%. This clearly shows that, when comparing studies, due attention must be given to the definition of sarcopenia. We also identified four SNPs (*FTO* rs9939609, *ESR1* rs4870044, *NOS3* rs1799983 and *TRHR* rs7832552) associated with sarcopenia and this information might be used (with other data) to identify individuals at a higher risk of sarcopenia and facilitate early targeted intervention to offset that higher risk.

## Methods

**Participants.** Participants comprised 60- to 91-year-old Caucasian women ( $n = 307$ ;  $70.7 \pm 5.7$  years,  $66.3 \pm 11.2$  kg,  $1.60 \pm 0.06$  m; mean  $\pm$  SD). All participants were recruited from social groups, and subsequent word-of-mouth between participants. These social groups ensured the participants were either physically active at a recreational level (e.g. table tennis, walking groups, dance classes) and societally engaged (e.g. book groups, and discussion groups). All were independently living, ambulatory and had no history of severe muscle and bone issues such as osteoporosis or rheumatoid arthritis, nor cancer, cardiovascular-related diseases or nervous system disorders such as Alzheimer's, convulsions or epilepsy.

**Skeletal muscle mass index.** Skeletal muscle mass was quantified using Bioelectrical Impedance Analysis (BIA) (Model 1500; Bodystat, Isle of Man, UK). Although Dual-Energy X-ray Absorptiometry (DEXA) is commonly used for measuring appendicular lean mass index (ALMI) in sarcopenia studies<sup>8,84</sup>, BIA has been suggested as a valid and low-cost alternative for measuring Skeletal Muscle Mass Index (SMI) and is a common tool in larger population studies<sup>27,85</sup>. Indeed, a high correlation between muscle mass measured with BIA and DEXA has been reported<sup>78,79</sup>. Participants were instructed to lie on a physiotherapist bed in a supine position with both upper and lower limbs slightly abducted from the body for about 4–5 min. Two adhesive electrodes were placed on the dorsum of the hand and foot on the right side of the body. An electrical current (frequency: 50 kHz; amplitude: 0.4 mA) was then passed between these electrodes and the skeletal muscle mass was estimated using an established formula<sup>77</sup>:

$$\text{Skeletal Muscle Mass (SMM)} = [\text{Ht}^2/(\text{R} \times 0.401) + (\text{sex} \times 3.825) + (\text{age} \times -0.071)] + 5.102$$

Where Ht is height in cm, R is resistance in  $\Omega$  and age in years. For sex, a male is scored as 1 and female as 0. SMI as SMM/Ht<sup>2</sup> was calculated using SMM in kg and height of the participant in m.

**Handgrip strength.** Handgrip strength (HGS) was measured using a digital load cell handgrip dynamometer (JAMAR plus, JLW Instruments, Chicago, USA) with a previously validated protocol<sup>86</sup>. Participants were instructed to stand in an upright position with the dynamometer held with the arm straight, and flexed at 90° to the shoulder. Verbal encouragement was provided to each participant to squeeze the handgrip dynamometer with maximum force, which was maintained for 5 s. The left and right arm were alternated, with 1 min between trials. The highest grip strength of three maximal efforts on each arm was recorded. The test-retest reliability of HGS in the dominant hand is high (ICC = 0.99) in healthy elderly participants<sup>87</sup>.

**Physical activity scale for elderly questionnaire.** Participants completed the Physical Activity Scale for Elderly (PASE) questionnaire in the lab on the testing day. The questionnaire is a 7-day recall questionnaire identifying time spent undertaking activities such as sitting, moderate intensity activities, recreational activities, strenuous activities and endurance and muscle strength related exercises. The questionnaire includes questions related to time spent in household work, gardening, caring for a dependent person, and work (paid or voluntary). The total PASE score was computed by multiplying the amount of time spent in each activity (hours/week) or participation (yes/no) in an activity by the empirically derived item weights and summing over all activities<sup>88</sup>.

**DNA sample collection, DNA extraction, SNPs selection and genotyping.** Two techniques for DNA collection were adopted. All participants were encouraged to provide a forearm venous blood sample, of whom 189 did so. If participants were unwilling to provide a blood sample or there was difficulty in obtaining a blood sample, a saliva sample was collected (116 participants). It was not possible to collect either sample from two participants. Genotyping was successful for 99.9% of the sample-SNPs combinations (7,313 out of 7,320); four different assays did not work for four samples, and three for one sample.

Blood (5 mL) was collected from a superficial forearm vein in 5-mL EDTA tubes (BD Vacutainer Systems, Plymouth, UK). Samples were stored at  $-20^\circ\text{C}$  until further processing. Superfluous saliva samples were collected using Oragene DNA OG-500 collection tubes (DNA Genotek Inc., Ontario, Canada) according to the manufacturer's instructions. Saliva samples were stored at room temperature until DNA extraction. Genomic DNA was extracted from both blood and saliva samples using a QIAcube, QIAamp DNA Blood Mini kit and standard spin column protocol (Qiagen, Crawley, UK).

Extracted DNA samples were genotyped for 24 polymorphisms selected based on the literature on the previous associations of those SNPs with relevant phenotypes, previous or hypothesised associations with sarcopenia and understood functional relevance. An initially 36 candidate SNPs identified were then reduced to 24 considering the number of studies reporting each association, the presence of conflicting results and the known transcriptional difference for some of the SNPs. Associations of the SNPs with previously reported relevant phenotypes are presented in Supplementary Table S2.

Two techniques were adopted for genotyping. The Fluidigm EP1 system (Fluidigm, Cambridge, UK) was used initially, but where errors occurred ( $\sim 1\%$ ), such as when duplicate samples were not in agreement, a second run was performed using StepOnePlus real-time PCR (Applied Biosystems®, Paisley, UK). In brief, four runs were performed, and genotype was determined using Fluidigm 192.24 Dynamic Array IFC (Integrated Fluidic Circuit, Fluidigm) in accordance with the manufacturer's instructions. Each assay (4  $\mu\text{L}$ ) comprised 2.0  $\mu\text{L}$  of assay loading reagent [2X] (Fluidigm), 1.0  $\mu\text{L}$  SNP genotyping Assay Mix [40X] (Applied Biosystems®), 0.2  $\mu\text{L}$  ROX [50X] (Invitrogen, Carlsbad, CA) and 0.8  $\mu\text{L}$  DNA-free water (Qiagen). Each sample (4  $\mu\text{L}$ ) contained 1.6  $\mu\text{L}$  genomic DNA, 2.0  $\mu\text{L}$  GTXpress master mix [2X] (Applied Biosystems®, PN 4401892), 0.2  $\mu\text{L}$  Fast GT Sample Loading Reagent [20X] (Fluidigm, PN 100–3065), and 0.2  $\mu\text{L}$  DNA-free water. No-template controls (NTCs) were included in each run. Each of the assays (3.75  $\mu\text{L}$ ) and samples (4  $\mu\text{L}$ ) were pipetted into separate inlets of the chip according to manufacturer's instructions. Assays and samples were loaded and mixed using the Integrated IFC

Controller RX software. The chip was then loaded into a thermal cycler (FC1 Fluidigm, PN 100–1279 D1) and the GT 192.24 Fast v1.pcl protocol was run. The thermocycling protocol was: an initial 120 s at 95 °C followed by 45 cycles of denaturation for 2 s at 95 °C and then annealing and extension for 20 s at 60 °C. TaqMan assays included VIC<sup>®</sup> and FAM<sup>®</sup> dyes for all SNPs and genotypes were identified based on end-point fluorescence (<https://www.thermofisher.com/np/en/home.html>) (attached in Supplementary Table S3). When using the StepOnePlus, the reaction volume was 10 µL that contained 0.2 µL DNA, 5 µL GTXpress master mix, 4.3 µL nuclease-free H<sub>2</sub>O and 0.5 µL TaqMan SNP genotyping assay [20X]. An initial 20 s at 95 °C was followed by 50 cycles of denaturation for 3 s at 95 °C, then annealing and extension for 20 s at 60 °C. Genotypes were identified based on reporter dyes VIC<sup>®</sup> and FAM<sup>®</sup> intensity and visualized using cluster plots. Except for *PTK2* rs7460, for which nucleotides were reported on the reverse strand according to NCBI, others were reported on the forward strand (shown in Supplementary Table S3). All samples were analysed in duplicate and 100% agreement was required to minimise genotyping error<sup>89</sup>.

**Assessment of sarcopenia.** Sarcopenia was assessed using three different definitions. The first definition, %SMM, was calculated as SMM/body mass\*100. Participants were defined as sarcopenic if %SMM < 22.1%<sup>27</sup>. The second definition, SMI, previously used by Janssen *et al.*<sup>28</sup>, was calculated as SMM/height<sup>2</sup>. Participants were defined as sarcopenic if SMI ≤ 6.76 kg/m<sup>2</sup><sup>28</sup>. Definition three used the measures of low SMI and low HGS as suggested by the EWGSOP; for which individuals with both SMI < 6.76 kg/m<sup>2</sup> and a HGS < 20 kg were considered sarcopenic<sup>1</sup>.

**Statistical analysis.** The Kolmogorov-Smirnov test was used to assess whether the data had a normal distribution and Levene's test was used to assess the homogeneity of variance of HGS and SMI. The frequency distribution of each SNP was assessed for Hardy-Weinberg equilibrium (HWE) using chi-square tests. Binary logistic regression was performed to investigate the association of sarcopenia and the SNPs studied, with age and PASE score used as covariate. The analysis was performed separately for each individual SNP. In instances where the number of homozygous participants was low, the homozygous group was combined with the heterozygous group and a two-group analysis was performed.  $p < 0.05$  was considered statistically significant. Where there was an association or tendency of an association ( $0.05 < p < 0.15$ )<sup>90,91</sup>, the homozygous groups were combined with the heterozygous group in a recessive and dominant model and then the analyses were re-run. Odds ratios (OR) for the risk genotype for sarcopenia were estimated for each SNP. Benjamini-Hochberg correction was performed to reduce the chance of type I error for multiple testing<sup>92</sup> with 24 tests and False Discovery Rate of 0.25. All the tests were performed in SPSS Version 26.0.

**Ethics statement.** Study protocols were in accordance with the guidelines of the Declaration of Helsinki (World Medical Association, 2013) and approved by the Ethics Committee of Manchester Metropolitan University. Informed written consent was obtained from all participants prior to involvement in the study.

### Data availability

The datasets analysed during the present study are available on reasonable request from corresponding author.

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## Author contributions

P.K. and C.I.M. designed research; P.K. and L.H. performed research; P.K., G.S., G.O.P., H.D., A.W., M.T. and C.I.M. helped P.K. analysing and interpreting the results; P.K. wrote the manuscript; and L.H., G.S., G.O.P., H.D., A.W., M.T. and C.I.M. revised the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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