ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle 2021; **12**: 350–357
Published online 1 February 2021 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12658

Association of regular aerobic exercises and neuromuscular junction variants with incidence of frailty: an analysis of the Chinese Longitudinal Health and Longevity Survey

Yu-Jie Zhang[1†], Yao Yao[2†], Pei-Dong Zhang[1], Zhi-Hao Li[1], Pei Zhang[3], Fu-Rong Li[1], Zheng-He Wang[1], Dan Liu[1], Yue-Bin Lv[4], Lin Kang[5], Xiao-Ming Shi[4*] & Chen Mao[1*]

[1]*Department of Epidemiology, School of Public Health, Southern Medical University, Guangzhou, China,* [2]*Center for Healthy Aging and Development Studies, National School of Development, Peking University, Beijing, China,* [3]*School of Life Science, Beijing Institute of Technology, Beijing, China,* [4]*National Institute of Environmental Health, Chinese Center for Disease Control and Prevention, Beijing, China,* [5]*Department of Geriatrics, Peking Union Medical College Hospital, Beijing, China*

Abstract

Background Candidate genes of neuromuscular junction (NMJ) pathway increased risk of frailty, but the extent and whether can be offset by exercises was unclear. The aim of this study was to investigate the association between aerobic exercises and incident frailty regardless of NMJ pathway-related genetic risk.

Methods A cohort study on participants from Chinese Longitudinal Healthy Longevity Survey was conducted from 2008 to 2011. A total of 7006 participants (mean age of 80.6 ± 10.3 years) without frailty at baseline were interviewed to record aerobic exercise status, and 4053 individuals among them submitted saliva samples. NMJ pathway-related genes were genotyped and weighted genetic risk scores were constructed.

Results During a median follow-up of 3.1 years (19 634 person-years), there were 1345 cases (19.2%) of incident frailty. Persistent aerobic exercises were associated with a 26% lesser frailty risk [adjusted hazard ratio (*HR*) = 0.74, 95% confidence interval (*CI*) = 0.64–0.85]. This association was stronger in a subgroup of 1552 longevous participants (age between 90 and 111 years, adjusted *HR* = 0.72, 95% *CI* = 0.60–0.87). High genetic risk was associated with a 35% increased risk of frailty (adjusted *HR* = 1.35, 95% *CI* = 1.16–1.58). Of the participants with high genetic risk and no persistent aerobic exercises, there was a 59% increased risk of frailty (adjusted *HR* = 1.59, 95% *CI* = 1.20–2.09). *HRs* for the risk of frailty increased from the low genetic risk with persistent aerobic exercise to high genetic risk without persistent aerobic exercise (*P* trend <0.001).

Conclusions Both aerobic exercises and NMJ pathway-related genetic risk were significantly associated with frailty. Persistent aerobic exercises can partly offset NMJ pathway-related genetic risk to frailty in elderly people.

Keywords regular aerobic exercises; neuromuscular junction; frailty

Received: 15 April 2020; Accepted: 15 November 2020
**Correspondence to: Prof. Chen Mao, Department of Epidemiology, School of Public Health, Southern Medical University, Guangzhou 510515, Guangdong, China. Phone: (+86) 2061648430. Email: maochen9@smu.edu.cn; Prof. Shi Xiao-Ming, National Institute of Environmental Health, Chinese Center for Disease Control and Prevention, Beijing, China. Email:* shixm@chinacdc.cn
[†]*Yu-Jie Zhang and Yao Yao contributed equally to this work.*

Introduction

Both genetic and lifestyle factors, such as frequency of exercises, played roles in building up muscle. Mutants in *Agrin*, an activator on clustering of nicotinic acetylcholine receptors (nAchR) via muscle-specific tyrosine-protein kinase receptor/LDL receptor-related protein 4 (MuSK/LRP4) signalling, can cause early-onset myasthenia gravis.[1] Cases carried identical homozygous variants in the intracellular domain of MUSK were found to be dyskinesia.[2] Methylation of histone deacetylase (HDAC) 9 (HDAC9), conserved binding motifs of which on myogenin (*MyoG*) promotor, repressed transcription of *MyoG* and nAChR, which further caused neuromuscular junction (NMJ) misfunctions.[3,4] Calcineurin, encoded by *PPP3CA* and stabilized NMJ, was associated with endurance capacity and VO_2max trainability.[5,6] According to a previous study, NMJ pathway has been selected as 'hallmark of aging' by combined aging and longevity-related genes expression databases.[7] Thus, polygenic NMJ pathway risk scores combining multiple related alleles maybe a set of biomarkers in frailty and provide a quantitative measure of genetic frailty risk.

There was existing evidence that elderly people who were taking physical activities had a lower frailty risk,[8,9] but the certainty of evidence was not strong enough, and the optimal way remained uncertain.[10] It is possible that genetic risk can be offset by regular exercises in older people.[11] Even though molecular biology of aging researches suggested that exercises can moderate HDACs deacetylase activity towards myocyte enhancer factor 2 (MEF2), which interacted with myoblast determination protein (MyoD), synergistically activated *MyoG* expression,[12] little is known about the real-world role of exercises on genetic risk of older people, which involved NMJ pathway.

The aim of this study was to use data from a representative aging cohort with a certain number of longevous participants to investigate whether adherence to regular aerobic exercises can offset genetic risk caused by NMJ pathway-related variants for frailty.

Methods

Study design and participants

Baseline data were obtained from the Chinese Longitudinal Healthy Longevity Survey (CLHLS) 2008–2009 wave. CLHLS was a nationwide cohort study with randomly selected elderly participants from 23 provinces, which covered nearly 82% of the total population in China in 2010. The inclusion criteria were elderly people aged $\geq$60 years who accepted to participate in the survey. Participants were excluded if they had any of the following: (i) baseline frailty; (ii) incomplete key variables or lost to follow-up; (iii) death. The next check-up was in the 2011–2012 wave. A total of 7006 participants, of whom had their aerobic exercises status checked, were taken into analysis of the first step on the association between adherence to aerobic exercises and frailty. After samples with call rate $<$1, minor-allele frequency $<$0.1, the inbreeding coefficient $|F| > 0.1$ and identity by descent > 0.1 were excluded; 4053 who provided saliva sample and were willing to participate in genotyping assay were included in the analysis of the second part, whether NMJ pathway-related variants can increase the risk of frailty, and the third part, whether persistent aerobic exercises can offset the genetic risk arisen by NMJ variants to frailty. The flow chart can be seen in *Figure* 1.

All participants gave informed consent to take part in the study and for their data to be used. The study protocol was approved by Biomedical Ethics Committee of Peking University (IRB00001052-13074).

Assessment of aerobic exercises status

Aerobic exercises status was assessed by trained personnel at baseline based on two questions 'Do you exercise regularly at present?' and 'Did you exercise regularly in the past?'. The exercises referred to purposeful physical activities, such as walking, ball games, and running. Participants were regarded as 'Persistent aerobic exercises' if the answers of above-mentioned two questions were 'yes'.

Genotyping, imputation, and establishment of neuromuscular junction pathway risk score

Full details of genotyping, imputation and establishment of NMJ pathway risk score can be found in the supporting information. The genotypic data of *HDAC9*, *PPP3CA*, *MUSK*, and *MEF2A* were drawn from the CLHLS genome-wide association study dataset.[13] The variants were annotated by HaploReg v2. Single and multiple genotype–phenotype association analyses were performed by *Cox* regressions. Linkage disequilibrium (LD) was also checked by PLINK (1.06) before weighted NMJ pathway risk scores were constructed.

Ascertainment of frailty

Frailty was ascertained by frailty index (FI), which was created by health deficits, such as symptoms, signs, and disabilities. Variables were comprehensively constituted from Rockwood's Study and Gu Danan's Study about CLHLS.[14,15] Thirty-four variables were used to construct an FI (detail can be found in Supporting information, *Table* S1). Each variable was recoded as 0 (*absence of deficit*) or 1 (*presence*

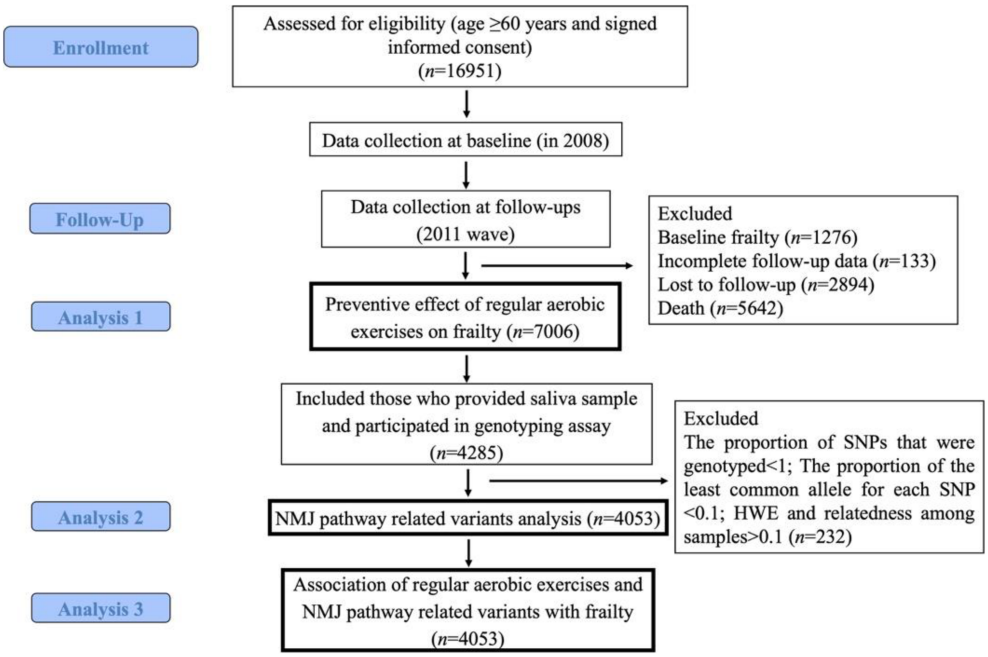

Figure 1 Flowchart of study population. Notes: SNP, single nucleotide polymorphism; HWE, Hardy–Weinberg equilibrium; NMJ, neuromuscular junction.

of deficit). For each individual, the FI score was calculated by summing up the deficits and divided by 34 (total number of items). Frailty was defined as FI $\geq$ 0.25.[16]

Statistical analysis

Genome-wide association study analysis was performed with R language packages, including snpStats, doParallel, SNPRelate, and GenABEL. Other data were analysed using Statistical Package for the Social Sciences (SPSS, Version 22.0, IBM Corp., Chicago, IL, USA).

All continuous variables with normal distribution were expressed as the *mean* (*standard deviation*). The variables with skewed distribution were expressed as the *median* (*interquartile range*). *Independent sample t test* for normally distributed variables was used to compare different groups. Categorical data were expressed as *count* (*percentage*). χ^2 tests were used to compare different groups of categorical data. *Cox regressions* were used to examine hazard ratios (*HRs*) of frailty identified by persistent aerobic exercises and NMJ pathway related homozygous variants. The *HRs* were adjusted for age, sex, smoking, drinking histories, years of schooling, body mass index (BMI), comorbidity (*Table* S2), living arrangement (whether alone), and high protein intake diversity (definition can be found in supporting information). Stratified analyses were performed to estimate potential modification effects on regular aerobic exercises according to age (60–79, 80–89, or 90–111), sex (men or women), low BMI [yes ($<$18.5 kg/m^2) or no ($\geq$18.5 kg/m^2)], one or more self-reported diseases (yes or no), balance on animal–plant proteins intake diversity (yes or no), and high dietary protein intake diversity (yes or no). Trend analysis was applied to explore the association between *HR*s of frailty and NMJ pathway-related genetic risk along with persistent aerobic exercise or not. A two-sided *P* value $<$0.05 was considered statistically significant. The related protein–protein interaction network was predicted by string analysis (https://string-db.org).[17] Conserved domains (CDS) of *MUSK* isoforms were checked in National Center for Biotechnology Information database.

Results

Participants' general characteristics

Table 1 summarizes the baseline characteristics of the participants. The 7006 participants had mean age of 80.6 ± 10.3 years, and 49.1% were men. The minimum age was 61 years, and the maximum age was 111 years. Based on Rockwood FI cut-off, during a total of 19 634 person-years (median [interquartile range] length of follow-up, 3.1 [2.9–3.2] years), there were 1345 cases (19.2%) of incident frailty. Frailty was significantly more prevalent in older age and in women than in men (all $P < 0.001$). Among the lifestyle factors, individuals with frailty had significantly lower proportions of persistent aerobic exercises (P = 0.034), and there

Table 1 Participants' clinical characteristics

	Non-frailty (n = 5661)	Frailty (n = 1345)	P value
Age (years)	78.7 (9.7)	88.7 (8.8)	<0.001
Sex			
Men (n, %)	2934 (51.8)	509 (37.8)	<0.001
Women (n, %)	2727 (48.2)	836 (62.2)	
Smoking (n, %)	1386 (24.5)	187 (13.9)	<0.001
Drinking (n, %)	1301 (23.0)	194 (14.4)	<0.001
Illiteracy (n, %)	2790 (49.3)	923 (68.6)	<0.001
Widowed (n, %)	1001 (17.7)	218 (16.2)	0.200
BMI (kg/m^2)	21.05 (3.57)	20.21 (3.56)	<0.001
Comorbidity (n, %)	1361 (24.0)	327 (24.3)	0.835
Dietary protein intake diversity (cat.)	2.6 (1.4)	2.6 (1.4)	0.626
Persistent aerobic exercises (n, %)	1239 (21.9)	259 (19.3)	0.034

BMI, body mass index.

were no significant differences in categories of dietary protein intake diversity (P = 0.626).

Association between persistent aerobic exercises and frailty

In whole population, compared with elderly people without persistent aerobic exercises, those with persistent aerobic exercises showed lesser frailty risk, with adjusted *HR* of 0.74 (95% *CI* = 0.64–0.85) (*Table* 2). This reverse association was stronger in a subgroup of 1552 longevous participants (age between 90 and 111 years, adjusted *HR* = 0.72, 95% *CI* = 0.60–0.87). The stratified analyses were uploaded in *Figure* S1.

Association between neuromuscular junction pathway-related variants and frailty

High NMJ pathway-related genetic risk was associated with a 35% increased risk of frailty in elderly individuals (adjusted *HR* = 1.35, 95% *CI* = 1.16–1.58, *Table* 2). Single-SNP analysis and LD distant analysis of each in the discovery sample of NMJ pathway variants related to frailty can be found in *Tables* S3 and S4, respectively.

Table 2 Risk of incident frailty according to persistent aerobic exercises and neuromuscular junction pathway-related genetic risk levels

	No. of participants	No. of cases of frailty	*HR* (95% *CI*)	P value
Persist aerobic exercises				
No	5508	1086	1.0 (Reference)	
Yes	1498	259	0.74 (0.64–0.85)	<0.001
Genetic risk				
Low	1932	271	1.0 (Reference)	
High	2121	394	1.35 (1.16–1.58)	<0.001

Notes: The *hazard ratio* of persist aerobic exercise was adjusted for age, sex, smoking, drinking histories, years of schooling, body mass index, comorbidity, whether alone and high protein intake diversity. Diseases were listed in Table S2.
CI, confidence interval; *HR*, hazard ratio.

Joint association of aerobic exercises and genetic risk with frailty

Of the participants with high genetic risk, persistent aerobic exercises were associated with a 25% lower risk of frailty (adjusted *HR* = 0.75, 95% *CI* = 0.57–0.99). Of the participants with no persistent aerobic exercises, high genetic risk was associated with a 40% increased risk of frailty (adjusted *HR* = 1.40, 95% *CI* = 1.17–1.67) (*Figure* 2). Of the participants with high genetic risk and no persistent aerobic exercises, there was a 59% increased risk of frailty (adjusted *HR* = 1.59, 95% *CI* = 1.20–2.09). *HRs* for the risk of frailty increased from the low genetic risk with persistent aerobic exercise to high genetic risk without persistent aerobic exercise (P trend <0.001, *Figure* 3). There was no significant interaction between genetic risk and regular aerobic exercises (P for interaction = 0.309).

Discussion

This study, to the best of our knowledge, was the first report of genetic risk caused by NMJ pathway variants to frailty in a relatively large elderly population, which can be partly offset by persistent aerobic exercises.

Many years ago, one classic study showed that 8 weeks of high intensity resistance training improved muscle mass and functional mobility among nine people with an average age of 90.3 years.[18] More recently, another study demonstrated that an 8 week low to moderate intensity resistance exercises increased muscle strength in 40 residents aged 90–97 years. What counts was that the authors reported no major adverse effects associated with the intervention despite the advanced age and frailty of the participants.[19] A recent meta-analysis in older adults (n = 28 523, mean age 74.2 years) also found that long-term exercises (≥1 year), independent of exercise frequency, improved physical function but were not associated with an increased risk of dropout due to health issues and mortality.[20] However, the validation of persistent aerobic exercises in oldest old to prevent frailty has not been fully proof in these studies. In our study, with a subgroup of 1552 longevous participants (age between 90 and 111 years), we found a significantly association between persistent aerobic exercises and frailty (*Figure* S1), which had larger sample size of nonagenarians and centenarians with a stronger statistical power.

Low dietary protein intake, especially when accompanied with low physical activity, has been linked to progressive loss

	No. of Participants	Cases of frailty	*Hazard Ratio* (95%*CI*)	*P* value
Low genetic risk				
No persist aerobic exercise	1489	206	1.0 [Reference]	
Persist aerobic exercise	443	65	0.89 (0.66-1.19)	0.425
High genetic risk				
No persist aerobic exercise	1631	321	1.0 [Reference]	
Persist aerobic exercise	490	73	0.75 (0.57-0.99)	0.028
No persist aerobic exercise				
Low genetic risk	1489	206	1.0 [Reference]	
High genetic risk	1631	321	1.40 (1.17-1.67)	<0.001
Persist aerobic exercise				
Low genetic risk	443	65	1.0 [Reference]	
High genetic risk	490	73	1.14 (0.81-1.60)	0.458

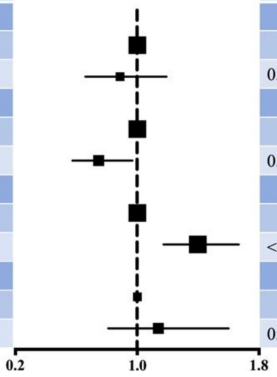

Figure 2 Risk of incident frailty according to NMJ pathway-related genetic risk levels or whether persisting aerobic exercises or not. Notes: NMJ, neuromuscular junction; the *hazard ratios* of persisting aerobic exercise were adjusted for age, sex, smoking, drinking histories, years of schooling, body mass index, comorbidity, whether alone, and high protein intake diversity. *CI*, confidence interval.

	No. of Participants	Cases of frailty	*Hazard Ratio* (95%*CI*)	*P* trend
Low genetic risk				<0.001
Persist aerobic exercise	443	65	1.0 [Reference]	
No persist aerobic exercise	1489	206	1.14 (0.85-1.52)	
High genetic risk				
Persist aerobic exercise	490	73	1.19 (0.85-1.67)	
No persist aerobic exercise	1631	321	1.59 (1.20-2.09)	

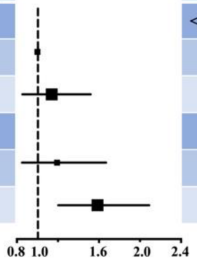

Figure 3 Risk of incident frailty according to NMJ pathway-related genetic risk levels and persisting aerobic exercises. Notes: NMJ, neuromuscular junction; the *hazard ratios* of persisting aerobic exercise were adjusted for age, sex, smoking, drinking histories, years of schooling, body mass index (BMI), comorbidity, whether alone, and high protein intake diversity. *CI*, confidence interval.

of muscle mass leading to low muscle strength, low walking speed, and feeling of exhaustion, all of which were associated with the physical frailty phenotype.[21–23] Indeed, several randomized controlled trials observed that the combination of physical activity and protein intake have a beneficial effect on muscle function and can help to prevent frailty.[24,25] But until now, far less is known on the association between appropriate protein intake, including protein source and timing, with physical activity and the broader concept of frailty.[26] In our study, with subgroups of 3574 participants who balanced on animal–plant protein intake diversity and 2068 who were in high dietary protein intake diversity, we found a stronger inverse relationship between persistent aerobic exercises and frailty (supporting information and *Figure* S1), which provided better evidence on positive effect of exercises in combination with protein intake on physical frailty.

Wnt signalling may contribute to impaired aged skeletal muscle repair. However, the role of Wnt signalling in impaired aged skeletal muscle repair is controversial. Some suggested that there was an exacerbation of Wnt signalling in aged skeletal muscle. Although Wnt signalling is required for completion of muscle repair, the incongruous elevation of Wnt signalling in aged skeletal muscle may result in dysfunctional myogenic capability and acceleration of aging. Besides, increased Wnt signalling in aged skeletal muscle may inhibit myogenicity.[27] On one side, one of Wnts family, Wnt 3a, was overactivated to up-regulate the expressions of p53,[28,29] a cell arrest protein, and significantly down-regulated MyoD1 and MyoG expression in muscular cells by activating HDAC9/MEF2, a transcription repressor on the MyoG promoter, which further impaired proliferation and differentiation of muscle progenitor cells and contributed to the development of muscle cell senescence and loss of muscle mass.[30,31] On the other side, overactivation of canonical Wnt/β-catenin signalling followed when soluble Wnt ligands interacted with postsynaptic membrane receptor, frizzled (one of MuSK CDS) and LRP4 on the muscle fibre.[32] Upon Wnt overactivation, signal transduced by segment polarity protein dishevelled homolog (DVL1) binding to C-terminus of frizzled, GSK3β's phosphorylation of β-catenin was activated, which did not allow nuclear translocation of β-catenin. Within the nucleus, inhibited β-catenin reduced

the binding to transcription factors such as T-cell factor 1 (TCF1) and lymphoid-enhancing factors 1 (LEF1), resulting in MyoD1 and MyoG inhibited.[33] Deprivation of calcineurin decreased MyoG expression by regulating MEF2, which possibly leads to abnormal NMJ formation.[6] Genetic variation in exons 10 and 18 of the *LRP5* modulates canonical Wnt signalling and the relationship between physical activity and bone mineral density in men. Wnt-LRP5 may play a role in the adaptation of bone to mechanical load in humans, which also implied the potential relationship between Wnt signalling and frailty.[34] In our study, we found that multiple variants, including the SNPs of *HDAC9*-rs2074633, *PPP3CA*-rs17030795, *MUSK*-rs2298492, and *MUSK*-rs2274419, increased risks of frailty. Clusters of HDAC9, calcineurin, and MuSK were linked to the core protein, wnt3a, a ligand for members of the frizzled family of seven transmembrane receptors, had functions in the canonical Wnt signalling pathway that associated with inhibition of MyoG, and participated in the modulation of chemical synaptic transmission (GO: 0050804, *Figure* S2). Especially, homozygous variant in rs2274419 changed encoded AA from Met to lle in frizzle domain of MuSK. The detailed analysis of rs2274419 location in CDS of different MuSK isoforms can be found in the supporting information and *Figure* S3. The possible reason was as follows: in the older people, even though variants of rs2074633, rs17030795, and rs2298492 happened in 3′utr or introns, which did not encode proteins, the synergistic effect, together with rs2274419 changed, may enlarge adverse impact of Wnts and further inhibit MyoD1 and MyoG in NMJ pathway, leading to muscle weakness, as a feature of frailty in muscular system.

The effect of exercises on several SNPs of genes associated with frailty has been reported before. Older individuals (aged 70 to 79 years) who exercised, with the angiotensin-converting enzyme (ACE) insertion/deletion (*DD*/*ID*) genotypes, were less likely to develop mobility limitation than those with the II genotype, which was associated with higher levels of intermuscular fat (IMF).[35] Another study showed that exercises reduced IMF in those aged between 50 and 83 years who are adrenergic receptor (ADR) β2 Glu27 carriers and Glu27 carriers who also carry the ADRα2b Glu9 allele.[36] To the SNP of TNFα-rs1800629, individuals aged over 65 years homozygous for the G allele presented a higher percentage of change with exercise training, with better functional performance in comparison with individuals with genotypes AA + AG. Besides, individuals with combined genotypes TNFα-rs1800629 GG and IL6-rs1800795 CC + CG showed greater improvement in physical performance after exercise training in comparison with other genotypes.[11] Middle-aged men (aged 50 to 60 years) with oestrogen receptor (ER) α (*ER*α) *PP* or *Pp* genotypes appear to have increased bone mineral density values in the lumbar spine after aerobic exercises training.[37] Catechol-*O*-methyltransferase (COMT) metabolized hydroxylation of oestrogens into more inactive methoxy-oestrogens, which would not bind to ER. Sedentary old women (aged 63 to 76 years) with the COMT polymorphism *HH* genotype had lower muscle mass, strength, and power, but they benefited the most from physical activity.[38] The α-actin3 gene (*ACTN3*) genotype exhibits a potentially modifying effect on muscle mass, maintenance of muscle function, and sarcopenia risk in elderly athletic populations, with the R allele associated with an increase in type-II muscle fibres.[39] Physically inactive participants (aged 50 to 85 years) with the IGF1 CA repeat polymorphism was significantly influenced on the change in strength and muscle volume combined with strength training.[40] But until now, whether persistent aerobic exercises can offset the risk of frailty partly caused by overactivated Wnt signalling in NMJ, especially at a very advanced age, was still unclear.[41] In our study, with a certain number of oldest old people, we found that, of the participants with high Wnt signalling-related genetic risk in NMJ, persistent aerobic exercises were associated with a partly lesser risk of frailty. However, little was known about the effects of exercises on Wnt signalling pathways in aged muscle, but because exercises elevated circulating pro-myogenic factors, it appeared reasonable to consider that appropriate aerobic exercises may release systemic factors that returned the balance of Wnt signalling in aged skeletal muscle.[42]

This study has several strengths. First, we reported a prospective association between persistent aerobic exercises and frailty, especially in nonagenarians and centenarians with a certain number. Second, we further proved that NMJ pathway was one of potential mechanisms in frailty. Third, we found that, of the participants with high Wnt signalling related genetic risk in NMJ, persistent aerobic exercises were associated with a lesser risk of frailty.

However, the work had several inevitable limitations. First, the results of genotyping assay were not identified by quantitative real-time polymerase chain reaction from human cells. Second, because saliva samples were voluntarily collected, people who did SNPs genotyping were not randomly assigned. Third, because of relatively small parts of elderly people persisting aerobic exercise, the function of persistent exercises in high genetic risk may be underestimated in the association analysis with frailty. Fourth, persistent aerobic exercises and dietary protein intake diversity were self-reported, which may arise recall biases. Fifth, the detailed varieties of sports and amount of foods were not recorded in this study. Sixth, although analyses were adjusted for a number of key confounding, such as age, sex, BMI, comorbidities, and protein intake diversity, the possibility of unmeasured determinants and reverse causation remained. Seventh, additional NMJ pathway variants associated with frailty are likely to be identified in future studies, and these variants may prove useful in further refining estimates of genetic risk. The validation of NMJ pathway risk scores also need to be reappraised in another ascertained population.

In summary, both aerobic exercises and NMJ pathway-related genetic risk were significantly associated with frailty. Persistent aerobic exercises can partly offset genetic risk caused by NMJ pathway variants to frailty. This is an important finding given that regular aerobic exercise was a personal and economical habit without major adverse effect that can be easily developed to cope with accelerating global burden of frailty in elderly people.

Author contributions

ZYJ contributed to the conception of the study design and drafted the manuscript. YY did the genotyping assays and provided the basic data. ZPD, LZH, ZP, and LFR helped with the interpretation of data and the statistical analysis. WZH, LD, LYB and KL helped with the revision of manuscript. SXM and MC participated in the design of the study and were the guarantors of the work. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank all participating patients. Besides, we specially appreciate the strong support of Prof. Yi Zeng from Peking University. The authors certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.

Conflicts of interest

The authors declare that they have no competing interests.

Funding

This work was supported by grants from National Key Research and Development Program of China (2018YFC2000400 to C.M.), Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (2019 to C.M.), the Construction of High-level University of Guangdong (G820332010, G618339167 and G618339164 to C.M.), the National Natural Science Foundation of China (81973109 to C.M. and 81903392 to Y. Y.), China Postdoctoral Science Foundation funded project (2019M650359 to Y.Y.).

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 List of items included in the frailty index.
Table S2 List of items included in the comorbidity.
Table S3 Single-SNP analysis in the discovery sample of NMJ pathway variants related to frailty.
Table S4 LD distant analysis of significant NMJ pathway variants related to frailty.
Figure S1 Preventive effects of regular aerobic exercises according to risk factor of incident frailty and protein intakes.
Figure S2 String analysis of NMJ pathway related allelic variations during frailty process.
Figure S3 Association between rs2274419 and Musk CDS.

References

1. Maselli RA, Arredondo J, Ferns MJ, Wollmann RL. Synaptic basal lamina- associated congenital myasthenic syndromes. *Ann N Y Acad Sci* 2012;**1275**:36–48.
2. Tan-Sindhunata MB, Mathijssen IB, Smit M, Baas F, de Vries JI, van der Voorn JP, et al. Identification of a Dutch founder mutation in MUSK causing fetal akinesia deformation sequence. *Eur J Hum Genet* 2015;**23**:1151–1157.
3. Zhang CL, McKinsey TA, Olson EN. Association of class II histone deacetylases with heterochromatin protein 1: potential role for histone methylation in control of muscle differentiation. *Mol Cell Biol* 2002;**22**:7302–7312.
4. Tang H, Goldman D. Activity-dependent gene regulation in skeletal muscle is mediated by a histone deacetylase (HDAC)-Dach2-myogenin signal transduction cascade. *Proc Natl Acad Sci U S A* 2006;**103**:16977–16982.
5. Jiang LQ, Garcia-Roves PM, de Castro Barbosa T, Zierath JR, et al. Constitutively active calcineurin in skeletal muscle increases endurance performance and mitochondrial respiratory capacity. *Am J Physiol Endocrinol Metab* 2010;**298**: E8–E16.
6. Chen HH, Tsai LK, Liao KY, Wu TC, Huang YH, Huang YC, et al. Muscle-restricted nuclear receptor interaction protein knockout causes motor neuron degeneration through down-regulation of myogenin at the neuromuscular junction. *J Cachexia Sarcopenia Muscle* 2018;**9**:771–785.
7. Cardoso AL, Fernandes A, Aguilar-Pimentel JA, de Angelis MH, Guedes JR, Brito MA, et al. Towards frailty biomarkers: candidates from genes and pathways regulated in aging and age-related diseases. *Ageing Res Rev* 2018;**47**:214–277.
8. de Labra C, Guimaraes-Pinheiro C, Maseda A, Lorenzo T, Millán-Calenti JC. Effects of physical exercise interventions in frail older adults: a systematic review of randomized controlled trials. *BMC Geriatr* 2015;**15**:154.
9. Giné-Garriga M, Roqué-Fíguls M, Coll-Planas L, Sitjà-Rabert M, Salvà A. Physical exercise interventions for improving performance-based measures of physical function in community-dwelling, frail older adults: a systematic review and meta-analysis. *Arch Phys Med Rehabil* 2014;**95**:753–769. e3.
10. Dent E, Martin FC, Bergman H, Woo J, Romero-Ortuno R, Walston JD, et al.

Management of frailty: opportunities, challenges, and future directions. *Lancet* 2019;**394**:1376–1386.
11. Pereira DS, Mateo EC, de Queiroz BZ, Assumpção AM, Miranda AS, Felício DC, et al. TNF-α, IL6, and IL10 polymorphisms and the effect of physical exercise on inflammatory parameters and physical performance in elderly women. *Age (Dordr)* 2013;**35**:2455–2463.
12. Menzies K, Auwerx J. An acetylation rheostat for the control of muscle energy homeostasis. *J Mol Endocrinol* 2013;**51**: T101–T113.
13. Zeng Y, Nie C, Min J, Liu X, Li M, Chen H, et al. Novel loci and pathways significantly associated with longevity. *Sci Rep* 2016;**6**:21243.
14. Bennett S, Song X, Mitnitski A, Rockwood K. A limit to frailty in very old, community-dwelling people: a secondary analysis of the Chinese longitudinal health and longevity study. *Age Ageing* 2013;**42**:372–377.
15. Gu D, Dupre ME, Sautter J, Zhu H, Liu Y, Yi Z, et al. Frailty and mortality among Chinese at advanced ages. *J Gerontol B Psychol Sci Soc Sci* 2009;**64**:279–289.
16. Rockwood K, Andrew M, Mitnitski A. A comparison of two approaches to measuring frailty in elderly people. *J Gerontol A Biol Sci Med Sci* 2007;**62**:738–743.
17. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res* 2017;**45**:D362–D368.
18. Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, Evans WJ, et al. High-intensity strength training in nonagenarians. Effects on skeletal muscle. *JAMA* 1990;**263**:3029–3034.
19. Serra-Rexach JA, Bustamante-Ara N, Hierro Villarán M, et al. Short-term, light- to moderate-intensity exercise training improves leg muscle strength in the oldest old: a randomized controlled trial. *J Am Geriatr Soc* 2011;**59**:594–602.
20. García-Hermoso A, Ramirez-Vélez R, Sáez de Asteasu ML, Martínez-Velilla N, Zambom-Ferraresi F, Valenzuela PL, et al. Safety and effectiveness of long-term exercise interventions in older adults: a systematic review and meta-analysis of randomized controlled trials. *Sports Med* 2020;**50**:1095–1106.
21. Kobayashi S, Suga H, Sasaki S. Three-generation Study of Women on Diets and Health Study Group. Diet with a combination of high protein and high total antioxidant capacity is strongly associated with low prevalence of frailty among old Japanese women: a multicenter cross-sectional study. *Nutr J* 2017;**16**:29.
22. Bartali B, Frongillo EA, Bandinelli S, Lauretani F, Semba RD, Fried LP, et al. Low nutrient intake is an essential component of frailty in older persons. *J Gerontol A Biol Sci Med Sci* 2006;**61**:589–593.
23. Coelho-Júnior HJ, Milano-Teixeira L, Rodrigues B, Bacurau R, Marzetti E, Uchida M, et al. Relative protein intake and physical function in older adults: a systematic review and meta-analysis of observational studies. *Nutrients* 2018;**10**:1330.
24. Tieland M, van de Rest O, Dirks ML, van der Zwaluw N, Mensink M, van Loon LJ, et al. Protein supplementation improves physical performance in frail elderly people: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* 2012;**13**:720–726.
25. Bauer JM, Verlaan S, Bautmans I, Brandt K, Donini LM, Maggio M, et al. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* 2015;**16**:740–747.
26. Schoufour JD, Overdevest E, Weijs PJM, Tieland M. Dietary protein, exercise, and frailty domains. *Nutrients* 2019;**11**:2399.
27. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* 2007;**317**:807–810.
28. Lee KH, Li M, Michalowski AM, Zhang X, Liao H, Chen L, et al. A genomewide study identifies the Wnt signaling pathway as a major target of p53 in murine embryonic stem cells. *Proc Natl Acad Sci U S A* 2010;**107**:69–74.
29. Hu Z, Klein JD, Mitch WE, Zhang L, Martinez I, Wang XH, et al. MicroRNA-29 induces cellular senescence in aging muscle through multiple signaling pathways. *Aging (Albany NY)* 2014;**6**:160–175.
30. Méjat A, Ramond F, Bassel-Duby R, Khochbin S, Olson EN, Schaeffer L, et al. Histone deacetylase 9 couples neuronal activity to muscle chromatin acetylation and gene expression. *Nat Neurosci* 2005;**8**:313–321.
31. Ridgeway AG, Petropoulos H, Wilton S, Skerjanc IS, et al. Wnt signaling regulates the function of MyoD and myogenin. *J Biol Chem* 2000;**275**:32398–32405.
32. Ferraro E, Molinari F, Berghella L. Molecular control of neuromuscular junction development. *J Cachexia Sarcopenia Muscle* 2012;**3**:13–23.
33. Tsivitse S. Notch and Wnt signaling, physiological stimuli and postnatal myogenesis. *Int J Biol Sci* 2010;**6**:268–281.
34. Kiel DP, Ferrari SL, Cupples LA, Karasik D, Manen D, Imamovic A, et al. Genetic variation at the low-density lipoprotein receptor-related protein 5 (LRP5) locus modulates Wnt signaling and the relationship of physical activity with bone mineral density in men. *Bone* 2007;**40**:587–596.
35. Kritchevsky SB, Nicklas BJ, Visser M, Simonsick EM, Newman AB, Harris TB, et al. Angiotensin-converting enzyme insertion/deletion genotype, exercise, and physical decline. *JAMA* 2005;**294**:691–698.
36. Yao L, Delmonico MJ, Roth SM, Hand BD, Johns J, Conway J, et al. Adrenergic receptor genotype influence on midthigh intermuscular fat response to strength training in middle-aged and older adults. *J Gerontol A Biol Sci Med Sci* 2007;**62**:658–663.
37. Remes T, Väisänen SB, Mahonen A, Huuskonen J, Kröger H, Jurvelin JS, et al. Aerobic exercise and bone mineral density in middle-aged finnish men: a controlled randomized trial with reference to androgen receptor, aromatase, and estrogen receptor alpha gene polymorphisms. *Bone* 2003;**32**:412–420.
38. Ronkainen PH, Pöllänen E, Törmäkangas T, Tiainen K, Koskenvuo M, Kaprio J, et al. Catechol-*O*-methyltransferase gene polymorphism is associated with skeletal muscle properties in older women alone and together with physical activity. *PLoS One* 2008;**3**:e1819.
39. Pickering C, Kiely J. *ACTN3*, morbidity, and healthy aging. *Front Genet* 2018;**9**:15.
40. Hand BD, Kostek MC, Ferrell RE, Delmonico MJ, Douglass LW, Roth SM, et al. Influence of promoter region variants of insulin-like growth factor pathway genes on the strength-training response of muscle phenotypes in older adults. *J Appl Physiol (1985)* 2007;**103**:1678–1687.
41. Izquierdo M, Morley JE, Lucia A. Exercise in people over 85. *BMJ* 2020;**368**:m402.
42. Arthur ST, Cooley ID. The effect of physiological stimuli on sarcopenia; impact of Notch and Wnt signaling on impaired aged skeletal muscle repair. *Int J Biol Sci* 2012;**8**:731–760.