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

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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 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
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. Cases carried identical homozygous variants in the intracellular domain of MUSK were found to be dyskinesia. Methylation of histone deacetylase (HDAC) 9 (HDAC9), conserved binding motifs of which on myogenin (MyoG) promoter, repressed transcription of MyoG and nAChR, which further caused neuromuscular junction (NMJ) misfunctions. Calcinurin, encoded by PPP3CA and stabilized NMJ, was associated with endurance capacity and VO\textsubscript{max} trainability. According to a previous study, NMJ pathway has been selected as ‘hallmark of ageing’ by combined aging and longevity-related genes expression databases. 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, but the certainty of evidence was not strong enough, and the optimal way remained uncertain. It is possible that genetic risk can be offset by regular exercises in older people. 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, 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 ≥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. 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. 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...
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 ≥ 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); χ2 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/m²) or no (≥18.5 kg/m²)], 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 HRs 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
Aerobic exercises and NMJ variants with frailty

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/m²)                    | 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.

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. 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. A recent meta-analysis in older adults (n = 28523, 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. 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.
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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{27} On one side, one of Wnts family, Wnt3a, was overactivated to up-regulate the expressions of p53,\textsuperscript{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.\textsuperscript{30,31} On the other side, overactivation of canonical Wnt/\(\beta\)-catenin signalling followed when soluble Wnt ligands interacted with postsynaptic membrane receptor, frizzled (one of MuSK CDS) and LRP4 on the muscle fibre.\textsuperscript{32}

Upon Wnt overactivation, signal transduced by segment polarity protein dishevelled homolog (DVL1) binding to C-terminus of frizzled, GSK\(\beta\)'s phosphorylation of \(\beta\)-catenin was activated, which did not allow nuclear translocation of \(\beta\)-catenin. Within the nucleus, inhibited \(\beta\)-catenin reduced...
the binding to transcription factors such as T-cell factor 1 (TCF1) and lymphoid-enhancing factors 1 (LEF1), resulting in MyoD and MyoG inhibited. Deprivation of calcineurin decreased MyoG expression by regulating MEF2, which possibly leads to abnormal NMJ formation. Genetic variation in exons 10 and 18 of the LRPS modulates canonical Wnt signalling and the relationship between physical activity and bone mineral density in men. Wnt-LRPS 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. 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 Ile 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 MyoD 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). Another study showed that exercises reduced IMF in those aged between 50 and 83 years who were adrenergic receptor (ADR) j2 Glu27 carriers and Glu27 carriers who also carry the ADRa2b Glu9 allele. 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. Middle-aged men (aged 50 to 60 years) with oestrogen receptor (ER) a (ERα) PP or Pp genotypes appear to have increased bone mineral density values in the lumbar spine after aerobic exercises training. 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. 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. 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. 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. 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.

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 exercises, 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. SXW and MC participated in the design of the study and were the guarantors of the work. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that they have no competing interests.

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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.

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