Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations

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FOXO1A and FOXO3A are two members of the FoxO family. FOXO3A has recently been linked to human longevity in Japanese, German and Italian populations. Here we tested the genetic contribution of FOXO1A and FOXO3A to the longevity phenotype in Han Chinese population. Six tagging SNPs from FOXO1A and FOXO3A were selected and genotyped in 1817 centenarians and younger individuals. Two SNPs of FOXO1A were found to be associated with longevity in women (P = 0.01–0.005), whereas all three SNPs of FOXO3A were associated with longevity in both genders (P = 0.005–0.001). One SNP from FOXO1A was found not to be associated with longevity. In haplotype association tests, the OR (95% CI) for haplotypes TTG and CCG of FOXO1A in association with female longevity were 0.72 (0.58–0.90) and 1.38 (1.08–1.76), P = 0.0033 and 0.0063, respectively. The haplotypes of FOXO3A were associated with longevity in men [GTC: OR (95% CI) = 0.67 (0.51–0.86), P = 0.0014; CGT: OR (95% CI) = 1.48 (1.12–1.94), P = 0.0035] and in women [GTC: OR (95% CI) = 0.75 (0.60–0.94), P = 0.0094; CGT: OR (95% CI) = 1.47 (1.16–1.86), P = 0.0009]. The haplotype association tests were validated by permutation analysis. The association of FOXO1A with female longevity was replicated in 700 centenarians and younger individuals that were sampled geographically different from the original population. Thus, we demonstrate that, unlike FOXO3A, FOXO1A is more closely associated with human female longevity, suggesting that the genetic contribution to longevity trait may be affected by genders.

INTRODUCTION

Human longevity is a complex trait affected by both genetic and environmental factors (1,2). How the genetic determinants contribute to longevity has provoked great interest over the past decades. A twin-based study suggested that genetic components may account for ~25% of the variation in the longevity trait (3). Later, it was found that genetic factors have increasing effects, particularly after the age of 60 (4). Though longevity may not correlate perfectly with health

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status (5), a recent study has demonstrated that the offspring of centenarians have a lower risk of cardiovascular disease and live significantly longer than those of non-centenarians (6). This suggests that genetic contributions to longevity are, at least in part, due to protective effects against disease. Therefore, the search for longevity-associated genes should lead not only to an understanding of the fundamental mechanisms of human aging but also to the identification of new potential targets beneficial for medical care.

Despite growing evidence for genetic determinants of longevity, the identification of longevity-predisposing loci or genes remains very difficult because of strong environmental effects and the complexity of the longevity trait. Previously, genome-wide linkage analysis provided a link between the marker D4S1564 at chromosome 4 and the exceptional longevity phenotype (7,8). However, this locus has been debated in a recent study (9). Several loci or genes, except for APOE, were found to be associated with longevity in some studies, but not reproduced by others (10). A recent genome-wide association study in the Framingham population suggested that eight SNPs are strongly associated with the age of death. Among these eight, two SNPs, rs4943794 and rs10507486 located in the FOXO1A gene, present the strongest association tested in the generalized estimating equation (GEE) model (11). In addition, FOXO3A, the other FoxO member, has recently been associated with human longevity in Japanese, German and Southern Italian (12–14). FOXO1A and FOXO3A are strongly associated with the age of death. Among these eight SNPs, only females were collected. Body mass index (BMI) and systolic and diastolic blood pressure were about 1:3 in centenarians and 1.8:1 in controls in Population 1. But in Population 2, only females were collected. Body mass index (BMI) and systolic and diastolic blood pressures of both populations are presented in Table 1. The differences between case and control groups for the listed parameters were evaluated by Student’s t-test. A P-value <0.05 was considered statistically significant.

RESULTS

Characterization of population

Two pairs of cases (centenarians) and controls (younger individuals) were collected among the Han Chinese. One pair (Population 1) was from southern China whereas the other (Population 2) was from northern China. The mean ages of centenarians in Populations 1 and 2 were 102.3 ± 0.14 and 100.8 ± 0.19 years (mean ± SEM). The male-to-female ratio was about 1:3 in centenarians and 1.8:1 in controls in Population 1. But in Population 2, only females were collected. Body mass index (BMI) and systolic and diastolic blood pressures of both populations are presented in Table 1. The differences between case and control groups for the listed parameters were evaluated by Student’s t-test. A P-value <0.05 was considered statistically significant.

Single SNP association analysis

Six SNPs (rs17630266, rs2755209 and rs2755213 for FOXO1A; rs2253310, rs2802292 and rs4946936 for FOXO3A) were genotyped by direct sequencing in all selected individuals. No significant deviation from the Hardy–Weinberg equilibrium test was found for all six SNPs in both populations. In sex-combined centenarians and controls, except for SNP rs17630266, the minor allele frequencies (MAFs) of both rs2755209 and rs2755213 of FOXO1A were significantly lower in the centenarian group than in control.

Table 1. Characteristics of populations

<table>
<thead>
<tr>
<th>Items</th>
<th>Population 1 Case (n = 761)</th>
<th>Control (n = 1056)</th>
<th>P</th>
<th>Population 2 Case (n = 350)</th>
<th>Control (n = 350)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (m/f)</td>
<td>183/578</td>
<td>682/374</td>
<td>&lt;0.001</td>
<td>0/350</td>
<td>0/350</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (year)</td>
<td>102.3 ± 0.14</td>
<td>47.1 ± 0.22</td>
<td>&lt;0.001</td>
<td>100.8 ± 0.19</td>
<td>41.4 ± 0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.9 ± 0.2</td>
<td>24.9 ± 0.1</td>
<td>&lt;0.001</td>
<td>18.5 ± 0.3</td>
<td>21.3 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>146 ± 0.9</td>
<td>129 ± 0.5</td>
<td>&lt;0.001</td>
<td>149 ± 1.4</td>
<td>115 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82 ± 0.5</td>
<td>84 ± 0.3</td>
<td>0.010</td>
<td>83 ± 0.7</td>
<td>73 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SEM (standard error of the mean). P-values are calculated from t-test comparing case and control groups within population. Case, centenarian; Control: individuals from general population; m/f, male/female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Table 2. Allelic association of FOXO1A and FOXO3A with longevity (sex-combined in Population 1)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele (major/minor)</th>
<th>Function</th>
<th>MAF (case/control)</th>
<th>( P_{\text{HWT}} )</th>
<th>OR (95% CI)</th>
<th>( P )</th>
<th>bP</th>
<th>PW (( \alpha = 0.05 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17630266</td>
<td>G/T</td>
<td>Intronic</td>
<td>0.392/0.372</td>
<td>0.50</td>
<td>1.09(0.95–1.25)</td>
<td>0.221</td>
<td>0.221</td>
<td>NC</td>
</tr>
<tr>
<td>rs2755209</td>
<td>C/T</td>
<td>Intronic</td>
<td>0.242/0.286</td>
<td>0.86</td>
<td>0.80(0.69–0.93)</td>
<td>4.6 \times 10^{-3}</td>
<td>9.0 \times 10^{-3}</td>
<td>0.83</td>
</tr>
<tr>
<td>rs2755213</td>
<td>C/T</td>
<td>Intronic</td>
<td>0.352/0.420</td>
<td>0.53</td>
<td>0.75(0.65–0.86)</td>
<td>7.4 \times 10^{-5}</td>
<td>3.0 \times 10^{-4}</td>
<td>0.98</td>
</tr>
<tr>
<td>rs2253110</td>
<td>G/C</td>
<td>Intronic</td>
<td>0.303/0.244</td>
<td>0.70</td>
<td>1.35(1.16–1.57)</td>
<td>7.9 \times 10^{-5}</td>
<td>2.3 \times 10^{-4}</td>
<td>0.97</td>
</tr>
<tr>
<td>rs2802292</td>
<td>T/G</td>
<td>Intronic</td>
<td>0.302/0.240</td>
<td>0.74</td>
<td>1.36(1.18–1.60)</td>
<td>2.9 \times 10^{-5}</td>
<td>1.5 \times 10^{-4}</td>
<td>0.98</td>
</tr>
<tr>
<td>rs4946036</td>
<td>C/T</td>
<td>3’-UTR</td>
<td>0.283/0.221</td>
<td>0.40</td>
<td>1.40(1.19–1.64)</td>
<td>1.8 \times 10^{-5}</td>
<td>1.1 \times 10^{-4}</td>
<td>0.99</td>
</tr>
</tbody>
</table>

MAF, minor allele frequency; \( P_{\text{HWT}} \), \( P \)-value from Hardy–Weinberg equilibrium test; OR (95% CI), odds ratio with 95% confidence interval; P, \( P \)-value calculated from \( \chi^2 \) test; bP, corrected \( P \) by Bonferroni step-down method; PW, power to reach an alpha level of 0.05 with a given population size; NC, not calculated; 3’-UTR, 3-untranslated region.

Table 3. Gender effects on allelic association of FOXO1A and FOXO3A with longevity trait in Population 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>Male (case/control)</th>
<th>OR (95% CI)</th>
<th>( P )</th>
<th>bP</th>
<th>Female (case/control)</th>
<th>OR (95% CI)</th>
<th>( P )</th>
<th>bP</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17630266</td>
<td>0.357/0.360</td>
<td>0.99(0.77–1.27)</td>
<td>0.91</td>
<td>0.91</td>
<td>0.402/0.392</td>
<td>1.04(0.86–1.27)</td>
<td>0.680</td>
<td>0.680</td>
</tr>
<tr>
<td>rs2755209</td>
<td>0.245/0.281</td>
<td>0.83(0.63–1.09)</td>
<td>0.166</td>
<td>0.33</td>
<td>0.243/0.294</td>
<td>0.77(0.62–0.95)</td>
<td>0.014</td>
<td>0.028</td>
</tr>
<tr>
<td>rs2755213</td>
<td>0.371/0.423</td>
<td>0.80(0.63–1.03)</td>
<td>0.073</td>
<td>0.22</td>
<td>0.348/0.413</td>
<td>0.76(0.62–0.92)</td>
<td>0.005</td>
<td>0.020</td>
</tr>
<tr>
<td>rs2253110</td>
<td>0.327/0.248</td>
<td>1.47(1.13–1.90)</td>
<td>0.003</td>
<td>0.014</td>
<td>0.296/0.235</td>
<td>1.37(1.10–1.71)</td>
<td>0.004</td>
<td>0.022</td>
</tr>
<tr>
<td>rs2802292</td>
<td>0.328/0.242</td>
<td>1.53(1.17–1.98)</td>
<td>0.001</td>
<td>0.006</td>
<td>0.295/0.236</td>
<td>1.36(1.09–1.69)</td>
<td>0.005</td>
<td>0.016</td>
</tr>
<tr>
<td>rs4946036</td>
<td>0.298/0.225</td>
<td>1.46(1.11–1.91)</td>
<td>0.004</td>
<td>0.017</td>
<td>0.280/0.212</td>
<td>1.44(1.15–1.82)</td>
<td>0.001</td>
<td>0.008</td>
</tr>
</tbody>
</table>

MAF, minor allele frequency; OR (95% CI), odds ratio with 95% confidence interval; \( P \), \( P \)-value from \( \chi^2 \) test; bP, corrected \( P \) by Bonferroni step-down method.

The adjusted \( P \) for multiple comparison by the Bonferroni step-down procedure (bP) was from \( 9.0 \times 10^{-5} \) to \( 3 \times 10^{-4} \). Conversely, the MAFs of all SNPs of FOXO3A were much greater in the centenarian group than in control (bP = \( 2.3 \times 10^{-4} \). For the given sample size, the statistical power was 0.83–0.99 in the setting of an \( \alpha \) level of 0.05 (Table 2).

Because the sex ratios were significantly different between the centenarian and control, we compared the frequencies of each SNP between case and control within genders. In males, none of the three SNPs from FOXO1A showed a statistic difference in MAF distributions between the case and control groups; whereas the three SNPs from FOXO3A presented much higher MAFs in the centenarians than in the younger population (bP = 0.017–0.006) with a power of 0.79–0.89 (Table 3). In comparison of the female groups, two SNPs of FOXO1A, rs2755209 and rs2755213, had reduced MAFs in the centenarian group versus the younger group (bP = 0.028–0.02). The MAFs of all SNPs from FOXO3A were higher in centenarians than in the younger population in both genders (bP = 0.022–0.008, Table 3).

Genotype association analysis

To test the inheritance models through which FOXO1A and FOXO3A are associated with longevity, we performed a genotype association test with dominant, recessive and additive models using logistic regression with adjustment for BMI and SBP. For FOXO1A, two of its SNPs, rs2755209 and rs2755213, were associated with female longevity in a dominant and additive model (\( P = 0.01–0.005 \); Supplementary Material, Table S1). For FOXO3A, all three SNPs presented strong associations with longevity in both the dominant and additive models for males (\( P = 0.005–0.001 \)) and females (\( P = 0.005–0.0002 \); Supplementary Material, Table S1).

Linkage disequilibrium and haplotype association analysis

Linkage disequilibrium (LD) and haplotype blocks (\( D^2 \geq 0.90 \)) were defined and visualized by the solid spine of LD method (Supplementary Material, Fig. S1). The haplotypes with a frequency of \( \geq 5\% \) were subjected to an association test with longevity. We tested the distributions of seven selected haplotypes, five from FOXO1A and two from FOXO3A, in female centenarians and controls. The haplotypes TTG and CCG of FOXO3A as well as GTC and CGT of FOXO3A presented significant differences in their distributions between case and control with statistical power \( > 0.96 \). For FOXO1A, the frequency of haplotype TTG was significantly lower in centenarians than in controls, while CCG showed the opposite. A permutation test was used to correct multiple comparisons. The permutation \( P \)-values were 0.024 and 0.046, respectively (Supplementary Material, Table S2). For FOXO3A, GTC lost its statistical difference in permutation analysis. CGT appeared more frequently in centenarians than in controls, while CCG showed the opposite. A permutation test was used to correct multiple comparisons. The permutation \( P \)-values were 0.009; Supplementary Material, Table S2).

We then tested the association of haplotypes from FOXO3A with male longevity, and found that, opposite to CGT, GTC had a reduced frequency in centenarians compared with controls. Statistical powers for significant haplotype association in the \( \chi^2 \)-test were \( > 0.99 \). The statistical differences remained in the permutation test (Supplementary Material, Table S3).
Three hundred fifty female centenarians and 350 geographically matched younger female individuals were referred to as ‘Population 2’ (Table 1) and used for replicating the finding that FOXO1A is associated with female longevity. We found that the MAFs of SNPs rs2755209 and rs2755213 from FOXO1A were significantly reduced in the centenarian group compared with control (adjusted \( P = 0.025 \) and 0.009, respectively) while that of SNP rs17630266 was not distinct between the centenarian and control groups (Table 4). These results suggest that FOXO1A is associated with female longevity in Population 2.

### Effects of ages and ethnicities on the MAF distributions of longevity-associated SNPs and haplotypes

The ages when blood samples were collected were classified into three groups in both male and female. Decreased MAFs of SNPs rs2755209 and rs2755213 with age were found for FOXO1A in females (Supplementary Material, Table S4). On the other hand, the MAFs of all three SNPs from FOXO3A were enriched with increased age in both genders (Supplementary Material, Table S4). This enrichment was also reported for the SNPs from FOXO3A in Japanese and German longevity studies; which are in the same haplotypes and associated with the longevity trait (Table 5).

### DISCUSSION

In this study, we demonstrated that FOXO1A is strongly associated with human female longevity and validated a previous finding that FOXO3A is associated with the longevity phenotype in the Han Chinese population. The association of FOXO1A with male longevity is not statistically significant in our study. It may be possible that the small male centenarian population in this study does not allow to unveil a weak association between FOXO1A genotypes and male longevity. But it appears true that a female longevity trait is more susceptible to the genetic variations of FOXO1A.

That women live longer than men has been recognized. However, studies on identifying the genetic causes for this phenomenon are very limited and preliminary (30). Previously, Barbieri et al. (31) found that an exonic SNP replacing the amino acid Pro with Ala in peroxisome proliferators-activated receptor (PPAR) \( \gamma-2 \) has different distributions between long-lived males and controls (\( P = 0.035 \)), but not between female groups. Recently, it was found that an SNP (A/G)-308 in tumor necrosis factor has sex-dependent distributions, and allele A is specifically associated with male life expectancy (\( P = 0.019 \)) (32). In an animal model, it has been shown that the increased expression of \( dFOXO \) is associated only with female lifespan (21). Since \( D. \ melanogaster \) has only one form of FoxO(s), it is impossible to know which form of FoxOs in mammals could be responsible for female longevity. Our study identified that FOXO1A is strongly associated with human female longevity.
Over the past few years, whether FOXO1A is associated with human longevity has been debated (12,23,24). This is likely due, at least in part, to difficulties in collecting sufficient numbers of long-lived and well-controlled individuals from both genders who can be used for a genetic association study. To understand the possible reasons for the debate in the literature, we compared our study with others and found that, in addition to ethnicity, the sample sizes varied significantly. More importantly, we noted that none of the previous studies mentioned sexual dimorphism in the association of FOXOs with human longevity. The total numbers of centenarians were 761 in our original study and 350 in the replication, but only 122, 218 and 213 in the other three studies (12,23,24). It is possible that a small sample size, or improper sex combination, produces insufficient statistical power, therefore reducing the probability of identifying FOXO1A associated with human female longevity. Here, we took full advantage of the large centenarian populations to determine the association of FOXO1A and FOXO3A with longevity within genders. We validated the association of FOXO3A with human longevity previously found in Japanese, German and Italian population-based studies (12–14) and replicated the finding that FOXO1A is not associated with male longevity (12). More importantly, we demonstrate that FOXO1A is associated with female longevity, providing a new insight into how a genetic factor contributes to human longevity. The association of both FOXO1A and FOXO3A with the human longevity trait appears in additive and dominant models in Han Chinese. Interestingly, Han Chinese, Japanese, German and Italian share the same longevity-associated haplotypes of FOXO3A. This excludes the possible influences of population stratification on association studies.

FOXO1A and FOXO3A, members of the forkhead transcription factors of the FoxO family, serve as the direct downstream signaling molecules of AKT1 in insulin/insulin-like growth factor signaling pathways. In vivo, FOXO1A and FOXO3A regulate the cell cycle and growth, apoptosis, DNA damage responses and angiogenesis (33–36). Malfunctions of FOXO1A or FOXO3A are involved in various cancers, insulin resistance, altered immune responses and organ damage (15,22,37–40). In the cardiovascular system, for example, FOXO1A and/or FOXO3A are/is important for the onset of diabetic cardiomyopathy (41,42), cardiac hypertrophy (43,44) and ischemic heart disease (45,46). It is likely that FOXO1A and FOXO3A affect longevity through multiple pathways, such as insulin resistance, stress responses or proneness to disease. Earlier studies have provided several lines of evidence that both FOXO1A and FOXO3A are associated with HbA1c level and fasting plasma insulin (12), (47,48), suggesting that their contributions to human longevity may be due to balancing insulin sensitivity and insulin resistance through insulin/insulin-like growth factor signaling pathways. The question remains why FOXO1A is more closely associated with human female longevity.

It has been reported that insulin sensitivity is highly sex-differentiated at different developmental stages or under different stresses in both humans and animal models (49,50). In female rats, for example, a high-fat or high-sugar diet does not induce insulin-resistance as seen in males, indicating that females have a gender-dependent protective effect (50–53). But when suffering from diseases such as diabetes, the aged female shows more increased insulin resistance and susceptibility to ischemic injury in the heart than the male (54). These suggest that gender-related human longevity may be associated with sexual dimorphism in insulin resistance. Although FOXO1A and FOXO3A are both direct downstream molecules of AKT1 in insulin/insulin-like growth factor signaling pathways, their functions are not identical. For instance, mice without FOXO1A are embryonic lethal, but they are viable without FOXO3A, suggesting that FOXO1A is indispensable (35,55) and acts as a main factor mediating the insulin signaling pathway (56). Over-expression of dFOXO in D. melanogaster, an effect mimicking an impaired insulin signaling pathway, only protects females from paraquat, increasing their lifespan (21). This implies that FOXO1A plays a role in female longevity by regulating sex-dependent insulin sensitivity.

In addition, FOXO1A is highly expressed in the female reproductive system, including ovaries and uterus, whereas FOXO3A is more ubiquitously expressed in vivo. Several studies suggest that FOXO1A plays a major role in the regulation of female decidualization (57–59). In women, delayed menopause is associated with age of death. For instance, death is often postponed for a few more decades if menopause occurs after the age of 50 (60). It is also known in D. melanogaster that over-expression of dFOXO reduces female fecundity and extends lifespan (21). Thus, it will be of great interest to know whether FOXO1A affects female longevity by regulating reproduction. But whether this is sufficient to explain why FOXO1A shows association with human female longevity needs to be determined.

In summary, we demonstrate in this study that FOXO1A is associated with female longevity while FOXO3A is associated with longevity in both genders. The association of both FOXO1A and FOXO3A with the human longevity trait is inherited in additive or dominant fashions. Han Chinese shares the same longevity-associated haplotypes of FOXO3A with Japanese and German. Although the finding that FOXO1A is not associated with male longevity has been replicated in Han Chinese and Japanese populations, the numbers of male centenarians are relatively small in both studies. Therefore, it needs to be cautious. The association of FOXO1A and FOXO3A with human longevity needs to be validated in more ethnic groups and in larger populations.

MATERIALS AND METHODS

Study subjects
We performed the baseline survey of the CLHLS reported previously (61). In the Survey, we interviewed 9093 oldest-olds aged 80–116 with a questionnaire containing 404 questions and physical tests throughout 85% of the regions of China. Among these 9093, 8441 were Han Chinese. In this study, a total of 761 centenarians (long-lived group) and 1056 unre- lated younger individuals (control group) from southern China were selected and used as Population 1 for initial screening of all six SNPs. We also took 350 female centenarians and 350 younger individuals from northern China for the necessary replication of associations found in the initial
screening. All participants were Han Chinese. Finger-prick blood samples from centenarians were spotted onto S&S no. 903 filter paper (Schleicher & Schuell, Germany) and stored at 4°C after the spots were completely dried. Two to three milliliters of blood were obtained from each younger individual. Written informed consent was obtained from all participants or their representative family members in cases of centenarians who were incapable of signing. BMI was calculated based on the formula (body weight/height²). The basic characteristics of the studied populations are presented in Table 1. The study protocol was approved by the Institutional Review Board, Institute of Molecular Medicine at Peking University. The study conformed to the principles outlined in the Declaration of Helsinki.

Genotyping of SNPs

Human genomic DNA was isolated from a 5-mm diameter punch-out from each blood spot and EDTA-anticoagulated blood using the proteinase K methods described previously (62). Based on the HapMap (CHB+JPT), the tagging SNPs rs17630266, rs2755209 and rs2755213 were selected for FOXO1A, and rs2253310, rs2802292 and rs4946936 for FOXO3A. DNA fragments of 200–350 bp containing SNPs were amplified by PCR from 10 ng of genomic DNA from each participant, with the primers listed below. The amplified DNA fragments were purified and used for genotyping by direct-sequencing with a BigDye v1.1 kit and running on ABI 3130XL. Based on the Genbank numbers NT_024524 for FOXO1A and NT_027541 for FOXO3A, the pairs of PCR primers for amplification of rs17630266, rs2755209 and rs2755213 for FOXO1A as well as rs2253310, rs2802292 and rs4946936 for FOXO3A were 5′-GGTGATGCGACTGACATCT-3′/5′-GGGTCT-3′, 5′-GGTGATGCGACTGACATCT-3′/5′-GGGTCT-3′, and 5′-GGTGATGCGACTGACATCT-3′/5′-GGGTCT-3′, respectively.

Statistical methods

Allele and genotype frequencies for single SNPs were calculated and tested for departure from Hardy–Weinberg equilibrium using the χ² test. Differences in allele and genotype distribution between cases (centenarians) and controls (younger population) were analyzed using logistic regression adjusted for non-genetic covariates under various genetic models that were defined as 1 (aa + Aa) versus 0 (AA) for dominant, 1 (aa) versus 0 (AA + Aa) for recessive, and 0 (AA) versus 1 (Aa) versus 2 (aa) for additive (A: major allele; a: minor allele). A Bonferroni step-down method was used for multiple comparison correction in allele association tests (63). Linkage disequilibrium and haplotype blocks (D’ ≥ 0.90) were defined and visualized by the solid spine of LD method using Haplovie 4.0 (http://www.broad.mit.edu/haplovie/haplovie). The haplotypes with frequency ≥5% were subjected to an association test with longevity. The permutation P-value was obtained by simulating 100 000 times in haplotype association analysis (64).

The two-tailed P-values, odds ratios and 95% confidence intervals are presented for all association tests. Statistical power was calculated with the sampsi command in STATA (StataCorp LP) under a given sample size and significance level (α = 0.05).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

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Conflict of Interest statement. None declared.

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