



# The reduction of vascular disease risk mutations contributes to longevity in the Chinese population



Yong-Han He<sup>a,b,1</sup>, Xiang Lu<sup>a,b,1</sup>, Ming-Xin Bi<sup>c</sup>, Li-Qin Yang<sup>a,b</sup>,  
Liang-You Xu<sup>d</sup>, Qing-Peng Kong<sup>a,b,\*</sup>

<sup>a</sup> State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, The Chinese Academy of Sciences, Kunming 650223, China

<sup>b</sup> KIZ/CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming 650223, China

<sup>c</sup> Department of Nutrition and Food Hygiene, Public Health College, Harbin Medical University, 157 Baojian Road, Nangang District, Harbin 150081, China

<sup>d</sup> Dujiangyan Longevity Research Centre, Dujiangyan 611830, China

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## ABSTRACT

**Aim:** Genetic factors play important roles in determining human lifespan. Although some “longevity genes” have been identified to be implicated in human longevity, many disease-associated variants were also observed in the long-lived individuals. The oldest old and their offspring usually have a lower prevalence of age-related diseases, which is likely attributed to a reduction or an absence of disease risk variants.

**Methods and results:** To test this hypothesis, 23 disease risk single nucleotide polymorphisms (SNPs), identified by previous genome-wide association studies (GWASs), were selected and genotyped in 1074 samples consisting of 574 longevity subjects (over 90 years old) and 500 younger controls. Our results revealed that 5 SNPs (rs2144300, rs1864163, rs2200733, rs1967017, and rs7193343) displayed significantly lower allelic frequencies and odds ratios (ORs) in the longevity group than that in the control group. The frequencies of homozygous mutation genotypes and corresponding ORs of the rs1864163, rs2200733, rs127430, rs1967017, and rs12413409 were lower in the longevity subjects. Interestingly, most of the abovementioned SNPs

**Abbreviations:** AD, Alzheimer Disease; CHB, Chinese Han Beijing; CVD, cardiovascular disease; GWAS, genome-wide association study; MAF, minor allele frequency; OR, odds ratio; PD, Parkinson Disease; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

\* Corresponding author at: State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, The Chinese Academy of Sciences, Kunming 650223, China. Tel./fax: +86 871 65197967.

E-mail address: kongqp@mail.kiz.ac.cn (Q.-P. Kong).

<sup>1</sup> These authors contributed equally to this work.

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convey susceptibility to cardiovascular disease (CVD), which is the leading cause of deaths in old adults but shows a much lower incidence in the longevity individuals and their offspring.

**Conclusion:** Taking into account the observation that the longevity subjects and their offspring have lower rate of cardiovascular mortality, it is then most plausible that the lack of disease risk variants, especially the CVD, is a genetic contributor to longevity in the Chinese population.

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## Introduction

Human lifespan is influenced by multiple determinants, including various environmental and genetic factors. Though the non-genetic factors, such as diet, health habits, physical activity, and psychosocial factors are important, the role of heritability in determining human lifespan is attracting more and more attention. Age at death in adulthood has a heritability of approximately 25% (Murabito et al., 2012), and the heritability of living to at least 100, i. e. centenarians, has been estimated at 0.33 in women and 0.48 in men (Sebastiani and Perls, 2012). Epidemiological investigations reveal that the oldest old and their offspring usually have a delayed or reduced prevalence of age-related diseases, such as cardiovascular disease (CVD), Alzheimer Disease (AD), Parkinson Disease (PD), cancers and some metabolic diseases (Franceschi and Bonafe, 2003; Hitt et al., 1999; Terry et al., 2003; Terry et al., 2004), suggesting that the long-lived individuals may have some special genetic basis to help them to delay or escape these senile diseases.

In the past decade, a number of genes, e.g. *daf-2*, *daf-16* and *sir-2*, were discovered, in which some specific genetic alterations confer advantage in extending the organisms' lifespan (Kenyon et al., 1993; Lin et al., 1997; Tissenbaum and Guarente, 2001), indicating the existence of longevity genes. Only very few longevity genes are confirmed to be valid for human beings (Brooks-Wilson, 2013), which is difficult to explain the significantly reduced incidence of age-related diseases in the longevity subjects and their offspring. Alternatively, given the recognition that all common complex diseases increase with age, it is plausible that the low prevalence of age-related diseases in the long-lived people is attributed to a much lower frequency of risk alleles. Indeed, there is increasing evidence showing a lower frequency of disease risk alleles in the longevity subjects (Pinos et al., 2013; Ruiz et al., 2012; Schachter et al., 1994). However, inconsistent observation comes from a recent study on the age-related disease risk variants in the longevity subjects (Beekman et al., 2010), causing it highly controversial whether the long-lived people do contain a lower frequency of disease risk alleles. To provide more evidence, we selected 23 age-related disease risk variants with high prevalence and mortality rates in the elderly and had them genotyped in 1074 samples consisting of 574 longevity subjects (over 90 years) and 500 younger controls for the study. Our study reveals that the SNPs related to cardiovascular disease (CVD) show a much lower frequency in the longevity individuals than the controls, suggesting that the lack/scarcity of disease gene mutations could be a genetic contributor to longevity.

## Methods

### Subjects

total of 1074 Chinese subjects consisting of 574 longevity subjects (over 90 years, mean age 93.8 years) and 500 controls (mean age 51.7 years) were collected from Sichuan province of China in 2010. All of the longevity subjects had no severe life-threatening illness, such as heart attack, cerebellar hemorrhage, and cancer, according to the medical examination. Only some of them had decreased vision or hearing loss as reported previously (He et al., 2014; Ye et al., 2009). The control subjects were all healthy with no severe medical history. Blood samples for DNA isolation were obtained after a 12 h fasting period. The investigation conformed with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee at Kunming Institute of Zoology, Chinese Academy of Sciences. Written informed consent was obtained from each of the participants prior to the study.

### *Choice of SNPs, DNA isolation and genotyping*

The age-related diseases with high prevalence and mortality rate based on the surveys and statistics of the World Health Organization (WHO) are considered in the present study, and as a result 23 risk SNPs were chosen from the GWAS database (Table S1). The SNPs were selected according to the following criteria: 1) minor allele frequency (MAF) in Chinese populations  $\geq 20\%$  according to HapMap data in Chinese Han Beijing (Tables S2 and S3); 2) the SNPs were either C/T or A/G which is for being compatible with the genotyping system used (Beckman Coulter, Fullerton, CA, USA); and 3) SNPs located no matter where they are (coding gene, outside or in intronic regions). Total genomic DNA was isolated from peripheral EDTA blood samples using a standard phenol/chloroform method (Sambrook et al., 1989). Multiplex polymerase chain reaction (PCR) and SNP analyses were performed using the GenomeLab SNPstream Genotyping System (Beckman Coulter, Fullerton, CA) following the protocols as described by Valdes et al. (2006). Primers were optimally designed using a web-based software provided by Beckman Coulter (available at [www.autoprimer.com](http://www.autoprimer.com)) and were listed in Table S4.

### *Statistical analysis*

Differences in the genotypes and alleles between the longevity and control groups were compared using an online platform SHEsis (<http://analysis.bio-x.cn>) (Shi and He, 2005). For those SNPs with significantly different genotypic distributions, the  $\chi^2$  test was further used to analyze their associations with longevity by calculating the odds ratios (ORs). Bonferroni correction method for multiple comparisons was used where necessary. The significance threshold was set to observe P-value/number of comparisons (Morgan, 2007). For each polymorphism, the Hardy–Weinberg equilibrium was calculated using the gene-counting method, and differences were assessed using the  $\chi^2$  test. Statistical analyses were performed using SPSS statistical software version 13.0 (Beijing Stats Data Mining Co. Ltd.). P values less than 0.05 were considered statistically significant.

## **Results and discussion**

Human longevity is a complex phenotype with strong genetic predisposition. Based on the fact that the longevity individuals have a delayed or reduced prevalence of age-related diseases, we determined 23 SNPs in the longevity people over 90 years old. The selected SNPs are mainly focused on the age-related diseases with high prevalence, morbidity and mortality rates, such as CVD, cancers, neurodegenerative disorders, type 2 diabetes, hypertension and stroke (Table S1). After genotyping these SNPs, we identified 5 SNPs showing lower allelic frequencies and ORs in the longevity subjects than the controls, i.e. rs2144300, rs1864163, rs2200733, rs1967017, and rs7193343 (Table 1). Of them, the SNPs rs2144300, rs1864163, and rs1967017 were reported to be associated with CVD (Aulchenko et al., 2009; Willer et al., 2008; Yang et al., 2010); the SNPs rs2200733 and rs7193343 are the risk factors of ischemic stroke (Gudbjartsson et al., 2009; Shi et al., 2009). For genotypic distribution, our study observed 8 SNPs (rs501120, rs2144300, rs1864163, rs2200733, rs127430, rs1967017, rs7193343, and rs12413409) with significant differences between the longevity and control groups (Table 2). Further analysis showed that the SNPs rs1864163, rs2200733, rs127430, rs1967017, and rs12413409 had lower homozygous genotype frequencies and ORs in the longevity subjects than the controls (Table 3). Except for the SNPs (rs1967017, rs1864163, and rs2200733) indicated above, the rs127430 and rs12413409 are also two risk factors predisposing to CVD/myocardial infarction (Roberts and Stewart, 2012; Smith et al., 2010). In addition, the SNPs rs501120, rs2144300 and rs7193343 had lower frequencies of independent heterozygous genotype or a combination of heterozygous and homozygous genotypes as well as their corresponding ORs compared to the wild genotypes. Surprisingly, these 3 SNPs were also closely associated with CVD or stroke (Aulchenko et al., 2009; Gudbjartsson et al., 2009; Qi et al., 2011).

In agreement with the previous studies (Beekman et al., 2010; Mooijaart et al., 2011; Sebastiani et al., 2012), most of the SNPs considered here, such as type 2 diabetes and cancers, showed no distribution difference between the longevity and the younger controls. However, our results revealed that the risk SNPs with significantly lower frequencies in longevity cases were all related to vascular diseases, especially the CVD which is the leading cause of deaths worldwide in the elderly (Lopez et al., 2006; Writing Group et al., 2010). Consistently, Pinos et al. reported that rs1333049, a polymorphism which is closely associated with

**Table 1**

Allelic distributions of selected SNPs in the control and longevity subjects.

|            | Control     |             | Control number | Longevity    |              | Longevity number | Allelic analysis |       |             |                       |
|------------|-------------|-------------|----------------|--------------|--------------|------------------|------------------|-------|-------------|-----------------------|
|            | A           | B           |                | A            | B            |                  | $\chi^2$         | OR    | 95% CI      | P value               |
| rs4689388  | 920 (0.926) | 74 (0.074)  | 497            | 1059 (0.926) | 85 (0.074)   | 572              | 0.000            | 0.998 | 0.722–1.379 | 0.990                 |
| rs12425791 | 743 (0.749) | 249 (0.251) | 497            | 861 (0.753)  | 283 (0.247)  | 572              | 0.037            | 0.981 | 0.806–1.193 | 0.847                 |
| rs401681   | 308 (0.310) | 686 (0.690) | 497            | 395 (0.345)  | 749 (0.655)  | 572              | 3.024            | 0.851 | 0.710–1.021 | 0.082                 |
| rs2075650  | 81 (0.082)  | 911 (0.918) | 497            | 104 (0.091)  | 1040 (0.909) | 572              | 0.575            | 0.889 | 0.656–1.204 | 0.448                 |
| rs10821936 | 616 (0.620) | 378 (0.380) | 497            | 709 (0.620)  | 435 (0.380)  | 572              | 0.000            | 0.999 | 0.839–1.191 | 0.999                 |
| rs2300747  | 413 (0.415) | 581 (0.585) | 497            | 486 (0.425)  | 658 (0.575)  | 572              | 0.190            | 0.962 | 0.810–1.143 | 0.663                 |
| rs501120   | 356 (0.358) | 638 (0.642) | 497            | 403 (0.352)  | 741 (0.648)  | 572              | 0.080            | 1.026 | 0.859–1.225 | 0.777                 |
| rs2383208  | 447 (0.450) | 547 (0.550) | 497            | 502 (0.439)  | 642 (0.561)  | 572              | 0.255            | 1.045 | 0.881–1.240 | 0.613                 |
| rs6532197  | 671 (0.675) | 323 (0.325) | 497            | 790 (0.691)  | 354 (0.309)  | 572              | 0.591            | 0.931 | 0.776–1.117 | 0.442                 |
| rs947211   | 457 (0.460) | 537 (0.540) | 497            | 482 (0.421)  | 662 (0.579)  | 572              | 3.189            | 1.169 | 0.985–1.387 | 0.074                 |
| rs8034191  | 952 (0.958) | 42 (0.042)  | 497            | 1099 (0.961) | 45 (0.039)   | 572              | 0.116            | 0.928 | 0.604–1.426 | 0.733                 |
| rs17319721 | 898 (0.903) | 96 (0.097)  | 497            | 1035 (0.905) | 109 (0.095)  | 572              | 0.010            | 0.985 | 0.738–1.315 | 0.919                 |
| rs2144300  | 741 (0.747) | 251 (0.253) | 500            | 941 (0.820)  | 207 (0.180)  | 574              | 16.725           | 0.649 | 0.528–0.799 | $4.37 \times 10^{-5}$ |
| rs1864163  | 840 (0.845) | 154 (0.155) | 500            | 1010 (0.880) | 138 (0.120)  | 574              | 5.455            | 0.745 | 0.582–0.954 | 0.020                 |
| rs2200733  | 411 (0.459) | 485 (0.541) | 500            | 598 (0.521)  | 550 (0.479)  | 574              | 7.789            | 0.779 | 0.654–0.929 | 0.005                 |
| rs1735151  | 422 (0.422) | 578 (0.578) | 500            | 450 (0.392)  | 698 (0.608)  | 574              | 1.996            | 1.132 | 0.953–1.346 | 0.158                 |
| rs1902341  | 327 (0.328) | 669 (0.672) | 500            | 383 (0.334)  | 765 (0.666)  | 574              | 0.068            | 0.976 | 0.815–1.169 | 0.794                 |
| rs127430   | 352 (0.352) | 648 (0.648) | 500            | 428 (0.373)  | 720 (0.627)  | 574              | 1.002            | 0.914 | 0.766–1.090 | 0.317                 |
| rs1967017  | 667 (0.671) | 327 (0.329) | 500            | 861 (0.750)  | 287 (0.250)  | 574              | 16.249           | 0.679 | 0.563–0.821 | $5.61 \times 10^{-5}$ |
| rs11775334 | 771 (0.672) | 377 (0.328) | 500            | 644 (0.644)  | 356 (0.356)  | 574              | 1.811            | 0.885 | 0.739–1.058 | 0.178                 |
| rs7193343  | 630 (0.636) | 360 (0.364) | 500            | 780 (0.679)  | 368 (0.321)  | 574              | 4.393            | 0.826 | 0.690–0.988 | 0.036                 |
| rs12413409 | 585 (0.589) | 409 (0.411) | 500            | 719 (0.626)  | 429 (0.374)  | 574              | 3.192            | 0.853 | 0.717–1.016 | 0.074                 |
| rs10953541 | 820 (0.828) | 170 (0.172) | 500            | 986 (0.859)  | 162 (0.141)  | 574              | 3.795            | 0.793 | 0.627–1.002 | 0.051                 |

A, wild allele; B, disease risk allele; OR, odds ratio; 95% CI, 95% confidence interval; the number in parenthesis means the frequency of alleles.

**Table 2**

Genotypic distributions of selected SNPs in the control and longevity subjects.

|            | Control     |             |             | Control number | Longevity   |             |             | Longevity number | $\chi^2$ | P value               |
|------------|-------------|-------------|-------------|----------------|-------------|-------------|-------------|------------------|----------|-----------------------|
|            | AA          | AB          | BB          |                | AA          | AB          | BB          |                  |          |                       |
| rs4689388  | 424 (0.853) | 72 (0.145)  | 1 (0.002)   | 497            | 490 (0.857) | 79 (0.138)  | 3 (0.005)   | 572              | 0.833    | 0.660                 |
| rs12425791 | 271 (0.546) | 201 (0.405) | 24 (0.048)  | 497            | 322 (0.563) | 217 (0.379) | 33 (0.058)  | 572              | 1.017    | 0.602                 |
| rs401681   | 41 (0.082)  | 226 (0.455) | 230 (0.463) | 497            | 66 (0.115)  | 263 (0.460) | 243 (0.425) | 572              | 3.755    | 0.153                 |
| rs2075650  | 473 (0.827) | 94 (0.164)  | 5 (0.009)   | 497            | 4 (0.008)   | 73 (0.147)  | 419 (0.845) | 572              | 0.616    | 0.735                 |
| rs10821936 | 175 (0.352) | 266 (0.535) | 56 (0.113)  | 497            | 213 (0.372) | 283 (0.495) | 76 (0.133)  | 572              | 2.026    | 0.363                 |
| rs2300747  | 75 (0.151)  | 263 (0.529) | 159 (0.320) | 497            | 96 (0.168)  | 294 (0.514) | 182 (0.318) | 572              | 0.597    | 0.742                 |
| rs501120   | 44 (0.089)  | 268 (0.539) | 185 (0.372) | 497            | 83 (0.145)  | 237 (0.414) | 252 (0.441) | 572              | 18.983   | $7.55 \times 10^{-5}$ |
| rs2383208  | 81 (0.163)  | 285 (0.573) | 131 (0.264) | 497            | 98 (0.171)  | 306 (0.535) | 168 (0.294) | 572              | 1.686    | 0.430                 |
| rs6532197  | 209 (0.421) | 253 (0.509) | 35 (0.070)  | 497            | 265 (0.463) | 260 (0.455) | 47 (0.082)  | 572              | 3.222    | 0.200                 |
| rs947211   | 84 (0.169)  | 289 (0.581) | 124 (0.249) | 497            | 81 (0.142)  | 320 (0.559) | 171 (0.299) | 572              | 3.878    | 0.144                 |
| rs8034191  | 455 (0.915) | 42 (0.085)  | 0 (0.000)   | 497            | 529 (0.925) | 41 (0.072)  | 2 (0.003)   | 572              | 2.327    | 0.312                 |
| rs17319721 | 406 (0.817) | 86 (0.173)  | 5 (0.010)   | 497            | 466 (0.815) | 103 (0.180) | 3 (0.005)   | 572              | 0.901    | 0.638                 |
| rs2144300  | 268 (0.540) | 205 (0.413) | 23 (0.046)  | 500            | 386 (0.672) | 169 (0.294) | 19 (0.033)  | 574              | 19.555   | $5.67 \times 10^{-5}$ |
| rs1864163  | 370 (0.744) | 100 (0.201) | 27 (0.054)  | 500            | 445 (0.775) | 120 (0.209) | 9 (0.016)   | 574              | 12.247   | 0.002                 |
| rs2200733  | 109 (0.243) | 193 (0.431) | 146 (0.326) | 500            | 163 (0.284) | 272 (0.474) | 139 (0.242) | 574              | 8.915    | 0.012                 |
| rs1735151  | 75 (0.150)  | 272 (0.544) | 153 (0.306) | 500            | 64 (0.111)  | 322 (0.561) | 188 (0.328) | 574              | 3.589    | 0.166                 |
| rs1902341  | 53 (0.106)  | 221 (0.444) | 224 (0.450) | 500            | 67 (0.117)  | 249 (0.434) | 258 (0.449) | 574              | 0.313    | 0.855                 |
| rs127430   | 39 (0.078)  | 274 (0.548) | 187 (0.374) | 500            | 78 (0.136)  | 272 (0.474) | 224 (0.390) | 574              | 11.293   | $3.53 \times 10^{-3}$ |
| rs1967017  | 301 (0.606) | 65 (0.131)  | 131 (0.264) | 500            | 413 (0.720) | 35 (0.061)  | 126 (0.220) | 574              | 21.239   | $2.44 \times 10^{-5}$ |
| rs11775334 | 220 (0.440) | 204 (0.408) | 76 (0.152)  | 500            | 261 (0.455) | 249 (0.434) | 64 (0.111)  | 574              | 3.913    | 0.141                 |
| rs7193343  | 179 (0.362) | 272 (0.549) | 44 (0.089)  | 500            | 262 (0.456) | 256 (0.446) | 56 (0.098)  | 574              | 11.772   | 0.003                 |
| rs12413409 | 202 (0.406) | 181 (0.364) | 114 (0.229) | 500            | 223 (0.389) | 273 (0.476) | 78 (0.136)  | 574              | 21.003   | $2.75 \times 10^{-5}$ |
| rs10953541 | 343 (0.693) | 134 (0.271) | 18 (0.036)  | 500            | 425 (0.740) | 136 (0.237) | 13 (0.023)  | 574              | 3.759    | 0.153                 |

AA, wild genotype; AB, heterozygous genotype; BB, homozygous genotype; the number in parenthesis means the frequency of genotypes.

**Table 3**  
Genotypic associations of significant SNPs with longevity.

|            | BB vs. AA |       |             |         | AB vs. AA |       |             |         | AB + BB vs. AA |       |             |         |
|------------|-----------|-------|-------------|---------|-----------|-------|-------------|---------|----------------|-------|-------------|---------|
|            | $\chi^2$  | OR    | 95% CI      | P value | $\chi^2$  | OR    | 95% CI      | P value | $\chi^2$       | OR    | 95% CI      | P value |
| rs501120   | 2.412     | 0.722 | 0.478–1.090 | 0.120   | 13.780    | 0.469 | 0.313–0.703 | <0.001  | 8.130          | 0.572 | 0.389–0.843 | 0.004   |
| rs2144300  | 3.082     | 0.574 | 0.036–1.074 | 0.079   | 18.332    | 0.572 | 0.443–0.740 | <0.001  | 19.555         | 0.572 | 0.447–0.734 | <0.001  |
| rs1864163  | 12.138    | 0.277 | 0.129–0.579 | <0.001  | 0.000     | 0.998 | 0.740–1.346 | 0.988   | 1.389          | 0.845 | 0.638–1.119 | 0.251   |
| rs2200733  | 6.976     | 0.637 | 0.455–0.891 | 0.008   | 0.145     | 0.942 | 0.695–1.278 | 0.756   | 2.131          | 0.811 | 0.612–1.075 | 0.144   |
| rs127430   | 5.506     | 0.599 | 0.389–0.921 | 0.019   | 10.977    | 0.496 | 0.326–0.755 | 0.001   | 9.225          | 0.538 | 0.359–0.806 | 0.002   |
| rs1967017  | 5.947     | 0.701 | 0.527–0.933 | 0.015   | 18.496    | 0.392 | 0.254–0.607 | <0.001  | 15.544         | 0.599 | 0.463–0.773 | <0.001  |
| rs7193343  | 0.391     | 0.870 | 0.561–1.348 | 0.574   | 11.529    | 0.643 | 0.498–0.830 | 0.001   | 9.862          | 0.675 | 0.527–0.863 | 0.002   |
| rs12413409 | 7.427     | 0.620 | 0.439–0.875 | 0.007   | 5.241     | 1.366 | 1.046–1.785 | 0.025   | 0.358          | 1.078 | 0.843–1.378 | 0.550   |

AA, wild genotype; AB, heterozygous genotype; BB, homozygous genotype.  
ORs, odds ratios for genotypic contributions of SNPs in the longevity group compared to the control group; 95% CI, 95% confidence interval.  
The significance threshold ( $0.05/3 = 0.017$ ) was corrected by Bonferroni method for multiple comparisons.

coronary artery disease (CAD), showed a lower risk allelic frequency in Southern European centenarians as well. Although inconsistent result did have been reported elsewhere (Beekman et al., 2010), the proportion of selected CVD risk SNPs or different genetic backgrounds may account for the discrepancy. Nevertheless, taking into account our current results that longevity individuals lack or have infrequent risk alleles associated with the CVD, as well as the observation that the oldest old and their offspring have lower cardiovascular mortality (Terry et al., 2003; Terry et al., 2004), it is then most plausible that the lack of age-related disease risk variants, especially the CVD, serves as the genetic contributor to longevity in the Chinese population.

The limitation that should be noted is that some SNPs with significant association to phenotypes do not obey Hardy–Weinberg equilibrium, which may be caused by the assay nonspecificity or genotyping errors for the SNP (Hosking et al., 2004), however, which does not affect the conclusion due to that the remaining significant SNPs were in Hardy–Weinberg equilibrium and highly associated with the age-related diseases, especially the CVD. In addition, due to that the genotyping system can only recognize the C/T or A/G mutations, those SNPs with other mutation types were excluded from genotyping, so the criterion of SNP selection is another limitation for this study.

In conclusion, we genotyped 23 SNPs associated with age-related diseases in the longevity subjects, and found that the longevity individuals did lack some age-related disease risk variants, especially the CVD, which may contribute to longevity in the Chinese population.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.mgene.2014.09.010>.

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