

SCIENTIFIC REPORTS



OPEN

Novel loci and pathways significantly associated with longevity

Received: 25 August 2015

Accepted: 20 January 2016

Published: 25 February 2016

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Only two genome-wide significant loci associated with longevity have been identified so far, probably because of insufficient sample sizes of centenarians, whose genomes may harbor genetic variants associated with health and longevity. Here we report a genome-wide association study (GWAS) of Han Chinese with a sample size 2.7 times the largest previously published GWAS on centenarians. We identified 11 independent loci associated with longevity replicated in Southern-Northern regions of China, including two novel loci (*rs2069837-IL6*; *rs2440012-ANKRD20A9P*) with genome-wide significance and the rest with suggestive significance ($P < 3.65 \times 10^{-5}$). Eight independent SNPs overlapped across Han Chinese, European and U.S. populations, and *APOE* and *5q33.3* were replicated as longevity loci. Integrated analysis indicates four pathways (starch, sucrose and xenobiotic

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metabolism; immune response and inflammation; MAPK; calcium signaling) highly associated with longevity ($P \leq 0.006$) in Han Chinese. The association with longevity of three of these four pathways (MAPK; immunity; calcium signaling) is supported by findings in other human cohorts. Our novel finding on the association of starch, sucrose and xenobiotic metabolism pathway with longevity is consistent with the previous results from *Drosophila*. This study suggests protective mechanisms including immunity and nutrient metabolism and their interactions with environmental stress play key roles in human longevity.

Human longevity is a complex trait determined by both genetic and environmental factors, and the genetic influence increases with the higher ages^{1–4}. Prior studies showed that most centenarians compress disability period towards the ends of their lives, suggesting that they avoid diseases of aging and associated disability until old ages^{5–7}. Furthermore, the offspring of centenarians have significantly better health compared to peers^{8,9}. Hence, centenarian genomes may harbor genetic variants associated with health and longevity¹⁰. In recent years, a number of genome-wide association studies (GWAS) in North America and Europe have been conducted on longevity, but only two loci have been identified associated with longevity at a genome-wide significant level: the well-known *TOMM40/APOE/APOC1* locus which is negatively associated with longevity^{4,10,11}, and a second locus on chromosome 5q33.3, which was identified in a recent GWAS by Deelen *et al.*¹⁰.

While interest in this topic is strong, the field is hindered by a lack of databases with both genotypic and phenotypic information and sufficiently large sample sizes of centenarians^{4,11}. The largest sample size of centenarians in GWAS studies published to date was 801, in which the study found only one genome-wide significant single nucleotide polymorphism (SNP)⁴. Much larger samples of centenarians and ethnically matched controls are needed for genome wide significant discoveries of genetic associations with longevity^{3,11}.

To expand the catalogue of longevity-associated loci and gain a better understanding of the influences of genes and biological pathways on longevity, we performed a GWAS using the samples derived from the Chinese Longitudinal Healthy Longevity Survey (CLHLS) (section M1 of Methods). The present study involved 2,178 Han Chinese centenarians, which is about 2.7 times the largest sample size of ever-published GWAS on centenarians⁴, and 2,299 middle-age controls after sample filtering, implying reasonably good power in our study (section M2 of Methods).

Our work also represents the first GWAS of longevity in Asian populations and in developing countries more generally. There were about five centenarians per million in China in the 1990s, compared with 50 per million in Western Europe in the same period¹². Han Chinese centenarians may be more likely to have longevity-associated and/or disease-preventive genes than their centenarian counterparts in the West since they survived the brutal mortality regimes of the past when famine, wars, and starvation operated on birth cohorts of many millions. Unlike Western countries that received many international immigrants from other parts of the world resulting in relatively heterogeneous genetic compositions even within ethnic groups, China received very few international immigrants. Consequently, even though one might expect population substructures because of the long history of interaction with surrounding minority Chinese ethnic groups¹³, Han Chinese are relatively more homogenous in genetic composition compared to their Western counterparts. For example, it has been estimated that the average of genetic differences measured by F-statistics (F_{ST})¹⁴ between Han Chinese population samples ($F_{ST} = 0.002$) is much lower than that among European populations ($F_{ST} = 0.009$)¹³. Thus, GWAS with large samples of Han Chinese centenarians and middle-age controls are expected to be instrumental for identifying genetic variants related to longevity.

Results

Population characteristics and analytical strategy. Standard quality controls are detailed in section M2 of Methods. Based on existing literature on Chinese genetic studies¹⁵ and our principal component analysis (PCA) (section M4 of Methods, Supplementary Figs 1 and 2), we stratified the samples into two independent GWAS datasets of Southern and Northern regions, with 1,063/887 centenarians/controls in the Southern dataset, and 1,115/1,412 centenarians/controls in the Northern dataset (Supplementary Table 1). The genomic inflation factors (λ) in the Southern, Northern and combined datasets were 1.022, 1.010 and 1.022, respectively, indicating that the effects of population stratification on genetic analysis are well controlled (Supplementary Fig. 3)¹⁶. To minimize both false-positive and false-negative rates, we applied a novel bi-directional discovery-evaluation strategy¹⁷, which fully uses the two independent GWAS datasets of Southern and Northern regions by parallel analysis: one as the discovery dataset and the other as the evaluation dataset, and vice versa. As described in the section M5 of Methods, when analyzing two independent GWAS datasets, the classic uni-directional discovery-replication approach of assigning one GWAS dataset as discovery and another GWAS dataset as replication would result in a higher false-negative rate missing a substantial number of replicated SNPs that have a p-value higher than the threshold and lower than the nominal significance level in the discovery GWAS dataset but that reach the threshold significance level in the replication GWAS dataset. Our bi-directional discovery-evaluation strategy enables us to avoid the higher false-negative rate.

We defined *a priori* discovery threshold of $P < 10^{-4}$ as the discovery threshold p-value based on recent relevant literature as cited and discussed in section M5 of Methods. We used a nominal significance of $P < 0.05$ in the evaluation stage. We performed GWAS using logistic regression implemented in PLINK (version 1.06) and adjusted for the top two eigenvectors and sex (section M6 of Methods). Using the SNPs identified in the bi-directional discovery-evaluation analyses, we performed a combined analysis comparing Han Chinese 2,178 centenarians with 2,299 mid-age controls, adjusted for the geographic stratification of Southern and Northern regions in addition to sex. In addition, we performed a meta-analysis, treating the two independent datasets from

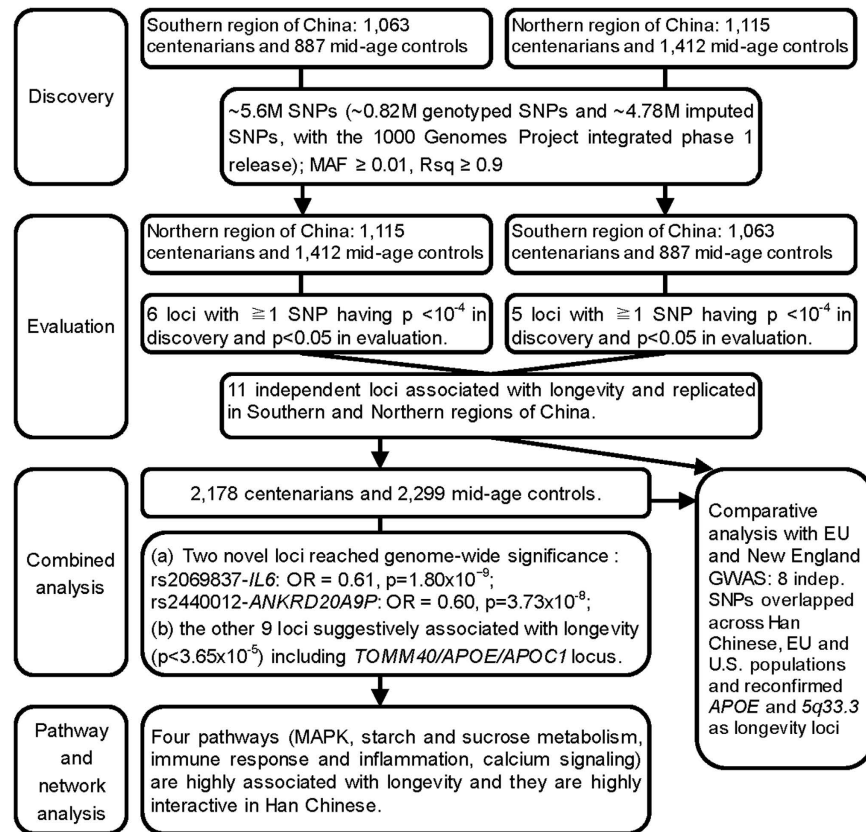


Figure 1. A flow chart of the consecutive analysis steps.

Southern and Northern regions of China as two groups. We then conducted an evaluation/comparison analysis of our results with the data from longevity GWAS of European Union (EU) longevity genetics consortium and the U.S. New England centenarians study (NECS). To extend the foregoing SNP association analyses towards understanding of biological processes underlying longevity, we conducted pathway and network analyses. A flow chart of the consecutive analysis steps is depicted in Fig. 1.

Association analyses of SNPs with longevity in Han Chinese. In the bi-directional discovery-evaluation analysis, we identified 11 independent loci (Table 1 and Fig. 2) that were replicated in the independent GWAS datasets of Southern and Northern regions of China. All 11 loci were associated with longevity with $p < 3.65 \times 10^{-5}$ in the combined dataset, of which two loci, rs2069837 (chromosome 7p15.3, *IL6*, $P = 1.80 \times 10^{-9}$) and rs2440012 (chromosome 13q12.12, *ANKRD20A9P*, $P = 3.73 \times 10^{-8}$) reached genome-wide significance ($P < 5 \times 10^{-8}$) (Table 1 and Fig. 2). *IL6* has been linked to longevity previously^{18,19}, but rs2069837 identified in this study is a novel signal within the *IL6* locus. The minor allele of rs2069837 is significantly less frequent among centenarians than middle-age individuals in Han Chinese (odds ratio = 0.61; $P = 1.80 \times 10^{-9}$), suggesting the effect of this locus on longevity is deleterious. This outcome is consistent with published findings that the *IL6* gene functions as an inflammatory biomarker of functional decline and poor health outcomes including increased mortality risk^{20,21}. The other novel SNP rs2440012 is located in *ANKRD20A9P*, a pseudogene that is affiliated with the long non-coding RNAs (lncRNA) class. The biological function of this particular non-coding transcript variant remains to be characterized.

Among the other 9 replicated loci associated with longevity at a suggestive significant level ($P < 3.65 \times 10^{-5}$), the *TOMM40/APOE/APOC1* locus is particularly interesting since its relationship with longevity is well known^{4,10,11}, and we discuss it in more detail below. The remaining 8 novel replicated loci include MIR3156-3 (rs145672791, 21q11.2, 28 kb downstream), *AKRIC2* (rs61856137, 10p15.1, 27 kb upstream), *FAM13A* (rs2704588, 4q22.1, intronic), *BEND4* (rs1487614, 4p13, 114 kb upstream), *EPHA6* (rs10934524, 3q11.2, 383 kb upstream), *ASIC2* (rs11658235 and rs7212444, 17q12, intronic) and *OLFM4* (rs9568833, 13q14.3, 200 kb downstream).

The meta-analysis results (last column in Table 1) are in full agreement with those of the combined analysis adjusted by a binary covariate of Southern and Northern regions, except the P values are slightly higher. Together, the 11 loci associated with longevity explained 3.38% of the variance in surviving to ages 100+ from middle-age, with each locus contributing from 0.39% (rs9568833-*OLFM4*) to 1.0% (rs2069837-*IL6*) of the variance based on effect estimates in the combined analysis, using the restricted maximum likelihood (REML) method²².

Notably, gender-specific association analysis for the 11 SNPs listed in Table 1 showed the same direction of effect in men and women with mostly very similar odds ratios (Supplementary Table 2). All of the 11 SNPs had

SNP	Chr.	Position	Nearest gene	Coded/ non-coded allele	Southern region of China			Northern region of China			Southern-Northern Combined			Meta analysis
					MAF (case/control)	P	Odds ratio	MAF (case/control)	P	Odds ratio	MAF (case/control)	P	Odds ratio	P
rs2069837	7	22768027	<i>IL6</i> (intronic)	G/A	0.018/0.033	5.98E-03	0.582	0.086/0.134	1.00E-06	0.64	0.053/0.095	1.80E-09	0.61	4.05E-08
rs2440012	13	19440123	<i>ANKRD20A9P</i> (nc_exonic)	G/C	0.050/0.092	1.38E-06	0.506	0.057/0.079	2.26E-03	0.69	0.054/0.084	3.73E-08	0.602	4.89E-08
rs145672791	21	14750023	<i>MIR3156-3</i> (28 kb downstream)	A/G	0.003/0.011	5.08E-03	0.267	0.004/0.022	9.88E-06	0.203	0.004/0.018	8.95E-08	0.219	2.34E-07
rs61856137	10	5087978	<i>AKR1C2</i> (27 kb upstream)	T/G	0.019/0.032	9.85E-03	0.572	0.040/0.070	1.56E-05	0.549	0.029/0.056	1.60E-07	0.544	7.54E-07
rs2704588	4	89849772	<i>FAM13A</i> (intronic)	C/T	0.004/0.013	4.32E-03	0.289	0.005/0.021	3.26E-05	0.237	0.004/0.018	2.38E-07	0.248	5.63E-07
rs1487614	4	42269480	<i>BEND4</i> (114 kb upstream)	T/C	0.112/0.146	1.85E-03	0.738	0.103/0.141	8.13E-05	0.707	0.107/0.143	2.87E-07	0.716	5.30E-07
rs10934524	3	96150160	<i>EPHA6</i> (383 kb upstream)	T/C	0.453/0.384	2.97E-05	1.354	0.470/0.431	4.76E-03	1.192	0.462/0.413	5.33E-07	1.266	1.16E-06
rs57681851	4	2290698	<i>ZFYVE28</i> (intronic)	G/T	0.187/0.136	7.05E-05	1.448	0.155/0.128	7.75E-03	1.256	0.170/0.131	1.83E-06	1.348	3.78E-06
rs7213812	17	31448649	<i>ASIC2</i> (intronic)	C/A	0.216/0.161	1.36E-05	1.45	0.176/0.152	2.84E-02	1.182	0.196/0.155	6.33E-06	1.29	6.25E-06
rs9568833	13	53827016	<i>OLFM4</i> (200 kb downstream)	T/C	0.145/0.193	7.85E-05	0.712	0.144/0.168	2.46E-02	0.836	0.144/0.177	1.77E-05	0.778	1.75E-05
rs405509	19	45408836	<i>APOE</i> (200 bp upstream)	G/T	0.374/0.316	7.92E-05	1.32	0.308/0.279	2.56E-02	1.148	0.341/0.293	3.64E-05	1.21	1.85E-05

Table 1. The 11 independent loci associated with longevity at $p < 10^{-4}$ in the discovery and at least a nominal significance ($p < 0.05$) in the evaluation, using the independent GWAS datasets of Southern and Northern regions of China as discovery/evaluation. Note: The SNPs rs57681851 and rs7213812 were imputed, while the other 9 SNPs listed in this Table were genotyped. MAF; minor allele frequency.

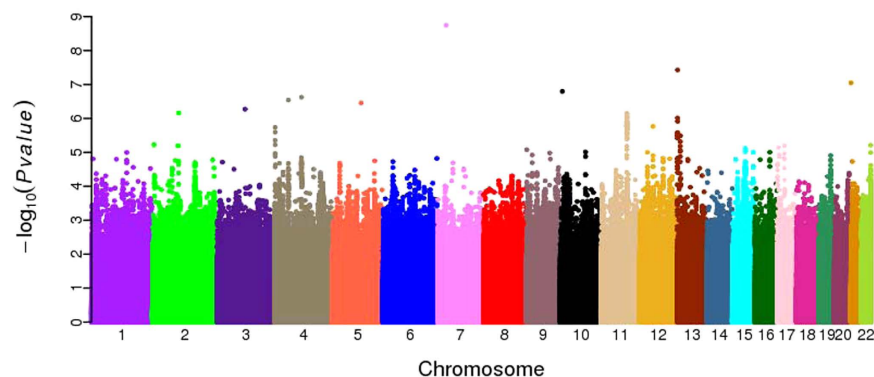


Figure 2. Manhattan plot showing the results of the association with longevity in the combined GWAS dataset.

a p -value of $p < 10^{-3}$ in women. Ten of the 11 SNPs achieved nominal significance ($P < 0.05$) in men and one SNP had a $P = 0.0549$ in men (Supplementary Table 1). The cross-gender similarities in effect direction/size on longevity of these 11 SNPs, which are replicated between discovery and evaluation datasets, is consistent with the results of the other GWAS on longevity^{10,4}, and the gender differences of the not-replicated SNPs is out of scope of this article and will be investigated in our subsequent study.

Comparison of current results with longevity GWAS studies of different ethnicities. To investigate the similarities and differences of genetic associations with longevity across the Han Chinese, European and U.S. ethnicities, we performed an evaluation/comparison analysis by using our Han Chinese GWAS as discovery and two European/American studies as evaluation. The two GWAS studies used for evaluation were the EU longevity genetics consortium, with a population of 5,406 aged 90+ and 15,112 controls aged <65 that included 14 studies from the Netherlands, Denmark, Iceland, Germany, Italy, the United Kingdom and Sweden¹⁰, and the U.S. New England centenarian study (NECS) of 1,030 long-lived individuals (age 95 and older for males and 100 and older for females) and 368 controls with a mean age of 79.9⁴.

The results presented in Supplementary Table 3 show that among the 11 SNPs associated with longevity and replicated in Southern and Northern GWAS datasets of CLHLS, six SNPs had available data in at least one of the two other longevity GWAS studies, and one SNP is not available in the other two longevity GWAS but it has

a proxy SNP ($r^2 = 0.97$) from the European population SNPs database (see Table 1 and its note (3)). The SNP rs405509 in *APOE* replicated in Southern and Northern GWAS datasets of CLHLS was also replicated in both EU ($P = 2.75 \times 10^{-06}$) and New England GWAS ($P = 2.46 \times 10^{-03}$), identifying a novel SNP-specific replication (Supplementary Table 3).

We conducted additional evaluation/comparison analysis of the other 723 SNPs associated with longevity at a suggestive significance level ($P < 10^{-4}$) in the Han Chinese GWAS combined dataset. Among the 723 SNPs, 267 were available in the EU GWAS, and 37 were available in the New England GWAS. Of these, eight independent SNPs associated with longevity ($P < 10^{-4}$) in the Han Chinese GWAS overlapped with at least nominal significance ($P < 0.05$) in at least one of the longevity GWAS in Europe (8 SNPs) and New England (2 SNP). Among these overlapped SNPs are rs405509 in *APOE* described above and rs4420638 in the *TOMM40/APOE/APOC1* locus, which was replicated across continents with strong supporting evidence in all of the three GWAS datasets: $P = 7.85 \times 10^{-5}$ in the Han Chinese, $P = 4.09 \times 10^{-21}$ in the EU and $P = 1.03 \times 10^{-09}$ in the New England.

In addition, we evaluated 54 SNPs associated with longevity ($P < 10^{-4}$) discovered in the four previously published major studies of GWAS on longevity^{4,10,11,23} and found that 44 of them were available in Han Chinese GWAS. Among these 44 SNPs, thirty-nine were not associated with longevity in the Han Chinese ($p > 0.05$, data not shown but available upon request), and four SNPs in the *TOMM40/APOE/APOC1* region reported in EU and New England GWAS replicated in the Han Chinese GWAS with $P < 10^{-4}$ (Supplementary Table 5). Furthermore, our Han Chinese GWAS identified an additional 10 SNPs with $P < 10^{-4}$ in the *TOMM40/APOE/APOC1* region; and among them, 8 SNPs were in very high linkage disequilibrium ($r^2 = 1.0$ or 0.99) with the significant SNPs reported in the EU and New England GWAS on longevity, and an additional 2 SNPs have not been reported before (Supplementary Table 5). Given the large number of associated SNPs in the *TOMM40/APOE/APOC1* region we performed a conditional analysis to identify independent association signals at this locus. The top independent association was rs405509 with $P = 3.64 \times 10^{-5}$. When conditioning on rs405509, we observed a secondary independent association at rs71352238 ($P_{\text{conditional}} = 2.1 \times 10^{-4}$) (Supplementary Fig. 4). After adjusting for rs405509 and rs71352238, we observed no other significant associations ($P > 0.01$) at this locus (Supplementary Fig. 4). These results demonstrate that the two independent associations in the *TOMM40/APOE/APOC1* account for all of the remaining associated signals in this region in the CLHLS GWAS.

The genome-wide significant longevity locus 5q33.3 (rs2149954, $P = 1.74 \times 10^{-8}$) reported in the EU GWAS¹⁰ showed the same direction of effect with $P = 0.02$ in the Han Chinese GWAS. We also found 4 SNPs of the reported longevity gene *FOXO3* are associated with longevity with a $P < 0.04$ and odds ratio around 1.2 in Han Chinese (data not shown, but available upon request).

The genome-wide significant locus identified in our Han Chinese GWAS, rs2069837 ($P = 1.80 \times 10^{-9}$), is located in the intronic region of the *IL6* gene on chromosome 7p15.3 (Table 1). The SNP rs2069827 in *IL6* that was reported previously as associated with longevity^{18,19} was not found in Han Chinese, but it is rather common in European populations based on 1000genome annotation in HaploReg V2. Similarly, our identified SNP rs2069837 has a MAF of 0.14 in Asian populations but 0.09 in the European population, indicating that the previously reported SNP rs2069827 is a European-specific longevity associated genotype and our identified SNP rs2069837 could be a Han Chinese-specific longevity associated genotype. The finding that these two genetic variants of *IL6* contribute to longevity in opposite directions in Han Chinese versus Europeans emphasizes the impact of genetic heterogeneity on longevity across ethnicities.

In brief, our comparative analysis indicates both considerable similarities and differences in genetic associations with longevity between the Chinese, European and U.S. populations. Further cross-national meta-analysis is warranted to develop an in-depth understanding of the cross-ethnic genetic associations with human longevity.

Pathway and network analyses. We conducted pathway analyses by applying i-GSEA4GWAS, an improved gene set enrichment analysis (GSEA) for GWAS²⁴ (section M7 of Methods). Twenty-five canonical pathways were ranked as significantly enriched (FDR < 0.05 and corrected p-value ≤ 0.004) and associated with longevity (Supplementary Table 6). These can be functionally classified into 4 major pathways: starch, sucrose and xenobiotic metabolism (10 enriched pathways), immune response and inflammation (7 enriched pathways), MAPK (4 enriched pathways) and calcium signaling (2 enriched pathways), plus 2 other significant enriched pathways (see Supplement-Table 6). Our finding of the four major significant pathways for longevity is generally similar to the results from Alzheimer's disease GWAS studies, in which over 20 genes with statistically-significant association signals²⁵ were mapped to relatively few major significant pathways including lipid metabolism, inflammatory response, endocytosis and immune response²⁶.

Among the four major pathways identified in our present study, p38 MAPK²⁷ and immunity²⁸ were linked to longevity previously. In particular, p38 MAPK acts downstream of the IL-6 receptor, and that may account for this association. p38 MAPK also acts upstream of FOXO, which, as discussed above, has been associated with longevity in previous GWAS. Moreover, we also note that p38 MAPK can be activated by AMPK, which is upstream of mTOR, and activation of mTOR has been shown to extend life span in *C. elegans*²⁹, mice³⁰ and yeast³¹. In common with our results, mTOR signaling was identified as significant for the ageing phenotype by a recent study³².

Before the current study, starch, sucrose and xenobiotic metabolism and calcium signaling had not been identified as being associated with human longevity in the European and American GWAS. The Chinese diet is high in carbohydrates, mainly starch and sucrose, and is usually low in fat. Sucrose is a heterodimer of glucose and fructose, while starch is a glucose polymer, and both molecules are degraded in the gut. The liberated glucose generates an insulin response. Fructose does not generate an insulin response but when it is consumed in excess it contributes to multiple chronic metabolic diseases. Fructose metabolism also can promote reactive oxygen species (ROS) formation, which leads to cellular dysfunction and aging³³. Thus, our novel finding that the starch, sucrose and xenobiotic metabolism pathway is significantly associated with human longevity in Han Chinese is interpretable, because the favorable defensive genotypes carried out by the Chinese centenarians may interact

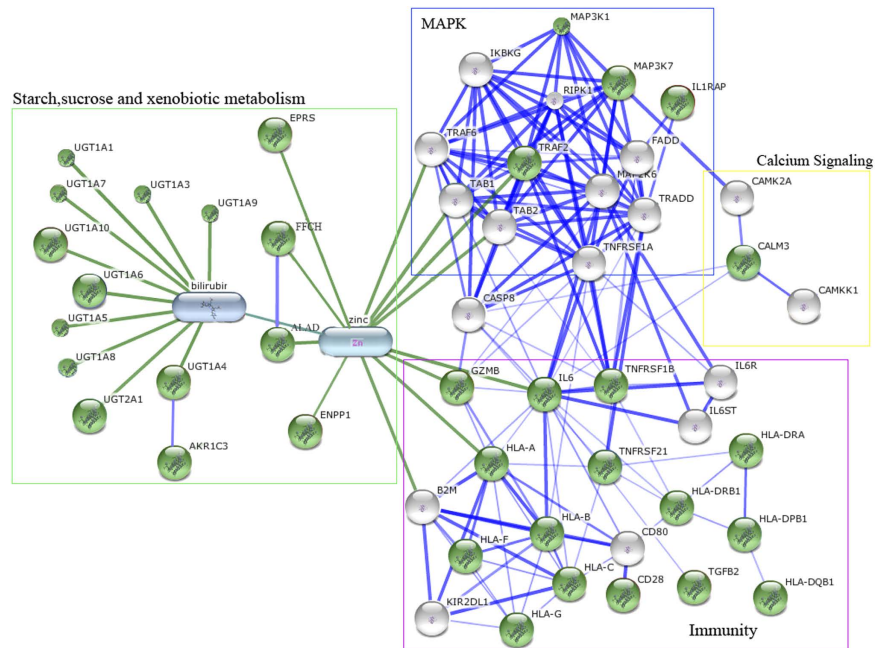


Figure 3. Gene and Pathway networks in longevity traits. Stronger associations are represented by thicker lines. Protein-protein interactions are shown in blue, protein-chemical interactions and chemical-chemical interactions in green. Note: The green nodes mark 35 highly-represented genes in the four main pathways, the white nodes mark highly-interacted genes with these 35 genes. The four main pathways are shown with colored rectangles.

with their high-carbohydrates diet to achieve extreme longevity. Furthermore, this novel finding is consistent with the hypothesis that dietary sources of carbohydrates, mainly sucrose and starch, may affect life span in *Drosophila*³⁴.

Altered calcium homeostasis contributes to neurodegenerative diseases of aging, including Alzheimer's disease (AD), and pharmacological inhibitors of calcium signaling have been shown to rescue structural plasticity defects in AD murine diseased neurons³⁵. Our analysis suggests that calcium signaling has a potentially important impact on prolonging longevity.

Noteworthy, support for the relevance to longevity of three of the four major significant pathways found in our Han Chinese GWAS (MAPK, immunity and calcium signaling) has been most recently provided by Tan *et al.*³⁶ as significant pathways in their longitudinal epigenome-wide association study (EWAS) of Danish twins with a mean age of 76 years at entry and, followed for 10 years. Interestingly, gene expression levels for the four significant pathways found in the Han Chinese GWAS were reported as differentially regulated during aging in *C. elegans*³⁷. These consistent findings of the important pathways for both genetic association with longevity as well as differential functional regulation over the aging process in both humans and animal models suggest that the functional coordination of these 4 major pathways have a profound impact on longevity and aging.

To further understand the interconnection of genes in the identified major pathways, we performed network analysis in STITCH³⁸ (section M8 of Methods) and identified 35 genes (Supplementary Table 7) that are highly represented across the 25 enriched pathways. These 35 highly-represented genes include gene family members *UGT1A* (the gene cluster that defends against organic molecules, such as small molecule toxins) and *HLA* (the gene functions in immunity protecting against pathogens), which indicate potential defensive mechanisms that are important for longevity. As shown in the connectivity map (Fig. 3), we find that the four major pathways that mediate defensive mechanisms and have a role in longevity, are highly interconnected. For example, *IL6*, a top ranked gene in both our SNPs and pathway analyses, is a key gene in immune response and inflammation and activating p38 via *IL6* receptor. The p38 MAPK pathway, in turn, mediates pathogen-specific responses by regulating expression of many protective genes related to immune response, contributing to longevity³⁹. Involvement of the both of *UGT1A* gene cluster, which detoxifies small molecules, and the *HLA* family, lend further support to the notion that defensive mechanisms are critical for successful longevity.

Taken together, our data strongly suggest that the longevity trait represents a complex interplay of multiple genes and pathways interacting with the environment, notably diet, that converge on specific biological processes.

Analysis of regulation of gene expression by associated variants. We examined the potential effects of the 11 SNPs or their proxies ($r^2 \geq 0.6$) in our study on gene expression using several eQTL databases (section M9 of Methods). Two SNPs in moderate LD ($r^2 = 0.61$) with rs11658235 (*ASIC2*), i.e. rs7224279 and rs11658301, showed significant trans-eQTL associations ($P = 3.88 \times 10^{-9}$) in human brain cerebellum tissue samples (Supplementary Table 9), but we did not find statistically significant eQTLs for other longevity-associated

variants. The findings of our SNP analysis and this eQTL analysis suggest *ASIC2* locus in brain cerebellum plays a role in mediating longevity phenotype.

We also investigated whether the SNPs with a significance level of $P < 10^{-3}$ in the 35 genes highly represented across pathways were associated with gene expression using eQTL data. By querying three publically available databases (GTEx, seeQTL, Chicago eQTL), we mapped multiple SNPs with highly significant cis-eQTLs in human tissue samples to three genes, including *MAP3K1*, *HLA-B* and *HLA-DPB1* (supplementary Tables 10–12).

Overlap between association signals and regulators of transcription. By querying the RegulomeDB database⁴⁰, which includes high-throughput, experimental datasets from ENCODE (Encyclopedia of DNA Elements data), we found rs405509 upstream of *APOE* exhibited a strong cis-effect on ZNF226 expression in lymphoblastoid cell lines⁴¹. Three SNPs in two other genes also mapped to regulatory regions, including rs2069837 in *IL6*, and rs7212444 and rs8064775 in *ASIC2*. These variants showed evidence of transcription factor binding and DNase I hypersensitivity peaks.

Discussion

Here, we summarize and discuss our key findings. First, our GWAS of Han Chinese identified 11 independent loci associated with longevity and replicated in two independent GWAS datasets from Southern and Northern regions of China (Table 1). More specifically, we identified two novel loci (rs2069837-*IL6* and rs2440012-*ANKRD20A9P*) that reached genome-wide significance, plus 9 suggestively significant loci ($P < 3.64 \times 10^{-5}$), including rs405509 in *APOE*, which is a well-known locus negatively associated with longevity⁴². Our strongest novel signal is for the rs2069837 SNP ($P = 1.80 \times 10^{-9}$) within the *IL6* locus at 7p15.3, negatively associated with longevity in the Han Chinese, which is consistent with previous findings that the *IL6* gene functions as an inflammatory biomarker of poor health outcomes^{20,21}. An earlier Dutch prospective cohort study of longevity reported a different SNP in *IL6*, rs2069827, which was positively associated with longevity¹⁸. The analyses showed that the previously reported SNP rs2069827 is European-specific and our newly identified SNP rs2069837 could be a Han-Chinese-specific longevity associated genotype. Another strong novel locus that reached genome-wide significance in Han Chinese GWAS was *ANKRD20A9P* (ankyrin repeat domain 20 family, member A9), a pseudogene which is affiliated with the lncRNA class. The biological function of this particular variant remains to be characterized.

Secondly, we identified 8 independent SNPs associated with longevity with $P < 10^{-4}$ in Han Chinese GWAS that overlapped results from the European and/or New England longevity GWAS, with at least nominal significance ($P < 0.05$). We also confirmed 4 previously reported longevity-associated SNPs and identified 2 independent associations with longevity in the well-known *TOMM40/APOE/APOC1* LD region. Our findings provided additional new signals and replicated the negative association of the *TOMM40/APOE/APOC1* LD region with exceptional longevity in Han Chinese, and confirmed 5q33.3 as a longevity locus that was previously identified in EU-GWAS. In short, we demonstrated both similarities and differentials in genetic associations with longevity across continents and ethnicities.

Thirdly, at the pathway level, our study identified 4 major pathways that influence longevity, including starch, sucrose and xenobiotic metabolism, immunity, MAPK signaling and calcium homeostasis. Among these pathways, p38 MAPK and immunity have been previously related to longevity in numerous studies^{27–31}. Our network analysis indicates these 4 identified pathways are highly interconnected. Collectively, our findings strongly support the notion that longevity is a polygenic trait influenced by a complex interplay of multiple genes and pathways. Interestingly, the three significant pathways (MAPK, immunity and calcium signaling) identified in our Han Chinese GWAS study were also most recently reconfirmed as significant pathways in the Danish longitudinal Epigenome-wide association study³⁶. Our novel finding that the starch, sucrose and xenobiotic metabolism pathway is significantly associated with human longevity in Han Chinese is consistent with the previous animal model study in *Drosophila*³⁴. Together, these data strengthen our findings that combinations of SNPs or genes represented among GWAS associations could converge upon gene pathways and biological processes related to healthy aging.

Like other GWAS studies on longevity, the major limitation of the present study is a lack of physiologically relevant model systems for further biological and functional validation of our new findings. This study represents the largest GWAS of centenarians to date and provides a particularly useful reference for analysis of disease mitigation or prevention genotypes, pathways and biological mechanisms. We believe that sharing the findings of this study will inspire biologists who are interested in aging research to conduct functional studies of the various identified genetic variants and pathways associated with longevity.

The findings in the present study support the hypothesis that defensive mechanisms (such as immunity) and metabolism driven by diet in response to environmental stress may play key roles in longevity in the Han Chinese. Diet mediated mechanisms also suggest that genetic and epigenetic influences upon extreme survival can differ according to culture and ethnicity. These findings may provide novel insights for expanding longevity theories that have emphasized genes associated with maintenance of genome integrity (especially DNA repair) and fertility-related mechanisms^{43–45}. Our new findings and previous studies about the reactive and adaptive nature of the immune system and about metabolism influenced by factors such as diet lead us to hypothesize that Han Chinese centenarians, who survived through the brutal past, may carry favorable defensive genotypes associated with longevity. Further studies are warranted for functional characterization of the new loci and interactive pathways to elucidate the underlying mechanisms for the observed associations with longevity.

Methods

M1. Samples and data source. DNA samples and data for the present GWAS are from the Chinese Longitudinal Healthy Longevity Surveys (CLHLS), which were conducted in 1998, 2000, 2002, 2005, 2008, 2011

and 2014 in a randomly selected half of the counties and cities in 22 out of 31 provinces in China. The CLHLS covers approximately 85% of the total population of China. We tried to interview all consented centenarians in the sampled counties and cities. For each centenarian interviewee, we recruited one nearby un-related middle-age control participant aged 40–59. “Nearby” is loosely defined – it could mean the same village or the same street if available, or in the same town or in the same sampled county or city⁴⁶. In the present study, all of the DNA samples of the middle-age controls were collected in the same county/city or different county/city but in the same province, as that of the centenarians.

Phenotype data were collected in the CLHLS using internationally standardized questionnaires adapted to the Chinese cultural and social context⁴⁶. Extensive evaluations of the data quality of the CLHLS, including assessments of mortality rate, proxy use, non-response rate, sample attrition, reliability and validity of major health measures, and the rates of logically inconsistent answers, have shown that the data from the CLHLS surveys are of good quality⁴⁷. The genetic samples and data from CLHLS were successfully used in prior published studies on candidate genes and gene-environment interactions relevant to longevity^{48–52}.

A wide variety of international and Chinese studies^{53,54} have confirmed that age reporting of the Han Chinese oldest-old aged 80+, including centenarians, is reasonably accurate; this is due to the Han Chinese cultural tradition of memorizing one’s date of birth to determine dates of important life events such as engagement, marriage, starting to build a residential house, etc. The accuracy of age reporting in the CLHLS data was reconfirmed by an investigation, which compared various standard demographic indices of age reporting among the oldest-old and age distributions of centenarians between the CLHLS and comparable data from Sweden, Japan, England and Wales, Australia, Canada, the U.S., and Chile; this study concluded that the age reporting of the Han Chinese oldest-old, including centenarians, is as good as the average in developed countries⁵⁵.

Note that the Han Chinese comprise about 93% of the total population in China, with 53 Chinese minority groups comprising 7% of the total population. The sample sizes of any minority group in the CLHLS data are too small for meaningful analysis, so we include Han Chinese samples only in the present study.

Descriptions of the samples and data sources of GWAS on longevity from the European Union Consortium and New England centenarians study (NECS) are given in the refs 4 and 10.

M2. Genotyping and quality controls. After DNA extraction, all of the 2,578 centenarians and 2,387 middle-age controls were genotyped using the Illumina HumanOmniZhongHua-8 BeadChips, which was created by strategically selecting optimized tag SNP content from all three HapMap phases and the 1000 Genomes Project (1 kGP). It allows profiling of 900,015 SNPs per sample, including 600 k SNPs of common variants (MAF \geq 5%), 290 k SNPs of rare variants (MAF < 5%) and 10 k SNPs existing only among Chinese and other Asian populations. In other words, 98.9% of the 900 k SNPs of the Illumina HumanOmniZhongHua-8 BeadChip are internationally compatible, with 1.1% specific to Chinese and Asian populations; this provides coverage of about 81% of common variants at $r^2 > 0.8$ with MAF \geq 5%, and about 60% of rare variants at $r^2 > 0.8$ with MAF < 5%. Our selection of this chip represents a state-of-the-art choice for GWAS in Asian populations to maximize international compatibility.

We randomly arranged cases and controls during sample batches, with negative controls (buffer water) and positive controls (YanHuang samples). For the sample filtering, 339 individuals for whom the genotypes were generated with a call rate less than 95% were excluded. We also computed identity-by-state probabilities for all subjects to search for any possible duplicates and kinship-related individuals among the samples, using PLINK (1.06)¹⁶. The 134 individuals who had identity-by-state probabilities with PI_HAT > 0.25 were excluded based on the IBD analysis implemented in PLINK.

To complete the sample filtering data quality control procedures and investigate population stratification in our GWAS samples, we performed principal components analysis (PCA) to evaluate genomic correlations in our dataset on the basis of pairwise identity by state (IBS) for all of the successfully genotyped samples using PLINK (1.06)¹⁶. We used the filter option (MAF > 0.01 and genotyping rate > 0.9) as discussed above; about 80% of the SNPs were used to calculate IBS. Our principle components analysis (PCA) showed that 9 individuals (all from Fujian province) were genetically separated from the total population of the 22 provinces (including Fujian) where the CLHLS was conducted, so these 9 individuals were excluded from our GWAS. Based on the PCA results, we also excluded 6 individuals who had serious deviations from the genetically-derived geographic membership in Southern or Northern populations.

After excluding these 15 outliers, our PCA showed that the cases and controls in this study were of Han Chinese ancestry and were well matched, without evidence of gross population stratification. In general, cases and controls were not separated on the basis of the first two principal components, and the cases and controls are evenly distributed in clusters of PC1 vs. PC2, PC1 vs. PC3 and PC2 vs. PC3 (see Supplementary Fig. 1). We computed the inflation factor (λ), a metric describing genome-wide inflation in association statistics⁵⁶, both before or after correcting for stratification using PLINK (1.06)¹⁶. Values of λ after correcting along 0, 1, 2, 3, or 4 eigenvectors are 1.109, 1.0241, 1.0235, 1.0226, or 1.0221, respectively, demonstrating that the correction is important and the top two eigenvectors correct nearly all of the stratification that can be corrected using the 4 eigenvectors. Consequently, in the subsequent GWAS regression models, we adjusted for the top two eigenvectors to minimize the effects of population stratification, following the approach adopted in the refs 54 and 57.

Finally, after various stages of sample filtering, 2,178 centenarians (mean age 102.7 ± 3.49 (SD)) and 2,299 middle-age controls (mean age 48.4 ± 7.44 (SD)) were included in the subsequent GWAS dataset (Supplementary Table 1). We also conducted quality-control filtering of the GWAS data from the total 4,477 individuals. Following the standard adopted in the published studies of GWAS using the same 900 k SNPs Illumina HumanOmniZhongHua-8 BeadChips⁵⁸ as the one used in present study, SNPs with call rates of less than 90% were removed from our GWAS dataset; SNPs were also excluded if they had a MAF less than 1% or if there was significant deviation from Hardy-Weinberg equilibrium in the samples, defined as $P < 10^{-5}$. SNPs on the X and

Y chromosomes and mitochondria were removed from the initial statistical analysis. Only 242 out of 743 (~30%) GWAS conducted from 2005 to 2011 considered chromosome X in their analyses according to the recent report of Wise *et al.*⁵⁹. Wise *et al.* discussed several reasons for exclusion of chromosome X, including a lower proportion of genes on chromosome X and a lower coverage of chromosome X on current genotyping platforms compared with autosomal coverage. They also described a number of technical hurdles that might add to the reluctance to include chromosome X in a GWAS, including complications in genotype calling, imputation, selection of test statistics, and the lack of readily available implementations. Accordingly, we excluded the chromosome X. After quality filtering and cleaning, 818,084 genotyped SNPs remained for association analysis in our final GWAS dataset.

M3. Imputation. To further increase genome coverage, we performed imputation analysis to infer the genotypes of all SNPs ($MAF \geq 0.01$) using IMPUTE software version 2⁶⁰ and the 1000 Genomes Project integrated phase 1 release as reference panel. SNPs with a quality score (Rsqr) of < 0.9 were discarded before analysis. After standard GWAS quality-control filtering for subjects and SNPs as described above, we obtained data for 5,595,657 SNPs (818,084 genotyped SNPs and 4,777,573 imputed SNPs) in 2,178 centenarians cases and 2,299 middle-age controls for the subsequent GWAS analyses.

M4. Stratifying Southern and Northern regions for our discovery-evaluation analysis. China is considered to have geographically and traditionally distinct Southern and Northern regions that have existed for a few thousand years, although there is no legal or official administrative meaning to this division. As reviewed in Xu *et al.*¹⁵, previous analyses of anthropological, anatomical, linguistic, and genetic data (including classic markers, microsatellite DNA markers, mtDNA, and Y chromosome SNP markers) have consistently suggested the presence of a significant boundary between the Southern and Northern populations in China. Among the 22 provinces where the CLHLS was conducted, Liaoning, Jilin, Heilongjiang, Hebei, Beijing, Tianjin, Shanxi, Shandong, Shaanxi, Jiangsu, Anhui and Henan belong to the geographically and traditionally defined Northern region; Shanghai, Zhejiang, Fujian, Jiangxi, Hubei, Hunan, Guangdong, Sichuan, Guangxi, Chongqing, and Hainan belong to the geographically and traditionally defined Southern region.

Our principle components analysis (described in section M2 above) also genetically confirmed the geographic and traditional cultural division between Southern and Northern regions in the Han Chinese GWAS dataset (Supplementary Fig. 2). Thus, we divided the entire sample into two independent GWAS datasets of Southern and Northern regions for our discovery-evaluation analysis. The Southern region dataset consists of 1,063 centenarians and 887 middle-age controls and the Northern region dataset consists of 1,115 centenarians and 1,412 middle-age controls (Supplementary Table 1). Given the nature of our population analysis and due to the fact that all of the samples analyzed in this study belong to the same ethnic group of Han Chinese living in one country with the same culture, we also performed a combined analysis comparing all centenarians with all controls. The genomic inflation factors (λ) in the Southern and Northern datasets and the combined dataset were 1.022, 1.010 and 1.022, respectively, suggesting that the association test statistics conformed to the underlying null distribution and would not require further adjustment for genomic control.

M5. A bi-directional discovery-evaluation strategy. In the classic uni-directional discovery-replication approach, the entire sample is divided into two datasets with one dataset used as discovery and the other dataset as the replication or valuation. The top SNPs found in the discovery stage using a pre-determined P value threshold are analyzed in the evaluation dataset. The SNPs with a P value lower than the threshold (e.g. $P < 1.0 \times 10^{-4}$) in the discovery stage and nominal significance (e.g. $P < 0.05$) in the evaluation stage are identified as significant/replicated SNPs, which are the final results of the analysis. This classic uni-directional discovery-evaluation approach is especially useful when the expensive GWAS serves as the first stage of discovery and the much less expensive second stage of evaluation genotypes only the top SNPs with a P value lower than the threshold found in the first stage. However, when analyzing two available independent GWAS datasets, the uni-directional discovery-evaluation approach of assigning one GWAS dataset as discovery and another GWAS dataset as evaluation would have a higher false-negative rate, missing a substantial number of significant/replicated SNPs which have a p -value higher than the threshold and lower than the nominal significance level in the discovery GWAS dataset but reach the threshold significance level in the evaluation GWAS dataset.

To avoid the high false-negative rate and to fully utilize all information in the two independent Southern and Northern GWAS datasets available to us, we applied a novel bi-directional discovery-evaluation strategy¹⁷ to search for consistent associations of SNPs with longevity. More specifically, the two GWAS datasets from Southern and Northern regions were analyzed with a parallel and bi-directional procedure: one is assigned as a discovery dataset and another as an evaluation dataset, and vice versa¹⁷. In our case, we first assigned and analyzed the Southern region GWAS dataset as discovery and Northern region GWAS dataset as evaluation, and then analyzed the Northern region GWAS dataset as discovery and Southern region GWAS dataset as evaluation (see Fig. 1). In this way, we capture all SNPs associated with longevity with a p -value lower than the threshold in the discovery stage and nominal significance in the evaluation stage.

The recent literature on GWAS discovery-evaluation analysis indicate that researchers defined a *priori* discovery threshold of $P < 10^{-5}$ or $P < 10^{-4}$, or $P < 10^{-3}$, depending on the circumstances of the research topics and the characteristics of the datasets used^{61–63}. In the present study of GWAS on longevity, we use exceptionally long-lived centenarians as cases and middle-aged adults as controls. Furthermore, we seek to identify SNPs which may individually have small effects, but may have large and important joint effects on the extremely complex trait of exceptional longevity. Thus, we believe that selecting the modest *a priori* discovery threshold of $P < 10^{-4}$ is a reasonable choice. More specifically, we defined the *a priori* discovery threshold of $P < 10^{-4}$ in the discovery stage in our bi-directional discovery-evaluation analyses using the independent GWAS datasets of Southern and

Northern rejoins of China, and in further international comparative analysis among the longevity GWAS datasets of the CLHLS, European Union and New England. We used a nominal significance level of $p < 0.05$ in the evaluation stage, following the usually adopted practice.

M6. Case-control study design and the statistical analysis. The logistic regression approach (named as “case-control association analysis on longevity”) or the “Fixed-Attributes Dynamics (FAD) Method”⁶⁴ and its extension⁴⁸ is often used to estimate the associations between genetic variants and longevity, through comparing long-lived individuals and middle-age controls^{4,10,11,51,52}. In fact, the “case-control association analysis on longevity” approach uses the same basic idea of FAD and produces exactly the same results of estimates as FAD does⁴⁸.

The method of case-control association analysis (or FAD) on longevity, which uses long-lived individuals as cases and ethnically/geographically matched middle-age persons as controls, is based on fundamental demographic insight that the prevalence of a genetic variant in a population can change with age even though no individuals can change their fixed attribute of the genetic variant, and that therefore much can be learned about the impact of the genetic variant on longevity. Ideally, we would use complete cohort data to compare the distributions of the genetic variants of members of the same cohort at two points of time in their life span: when they were young versus when some of them reach age 100+, while all of the other cohort members died before age 100. However, such long-term and complete follow-up GWAS or candidate gene genotype data for the members of the same birth cohort at young ages and ages 100+ are not currently available for any population. Thus, various previously published association studies on longevity used cross-sectional datasets to compare long-lived individuals (centenarians and/or nonagenarians) as cases and middle-aged as controls, observed in the same period. Such cross-sectional cases/controls analyses are based on two assumptions: (1) the initial distribution of the genetic variants does not differ substantially between the long-lived and middle-age cohorts, which is generally reasonable, because the basic genetic structure would not change substantially in about 50 years within the same ethnic population. (2) The basic genetic profiles of the non-migrants do not differ substantially from those migrants who are of the same ethnicity as non-migrants. This assumption is also generally reasonable, especially for studies of Han Chinese because China received remarkably few immigrants of other ethnicities in the past decades. With these two assumptions, one may intuitively understand that the proportion of genetic variants which are positively (or negatively) associated with longevity are significantly higher (or lower) among the centenarians, compared to the middle-aged controls, because those who have the favorite genetic profile have better chance to survive to age 100+, while those with less favorite genetic profile could not reach age 100. Such an intuitive mechanism based on the two assumptions outline above has been proven mathematically⁴⁸.

Note that in the middle aged controls, selective mortality is still very low and on the other hand it is impossible for natural selection to change the gene pool within a short period of several decades. Thus, genotype frequency in the middle-aged controls could well represent population genotypes. However, the findings based on the study design of using centenarians as cases and middle-aged as controls may be driven by the gene-environment interactions that lead to selective mortality for the centenarians. For example, the Chinese centenarians survived the brutal mortality regimes of the past when famine, wars, and starvation operated on their large birth cohorts. This may imply that these long-lived individuals who successfully passed through the hard life conditions and high mortality of earlier decades may carry genotypes that interact with environmental factors such as life stress, certain diet or behavior, which lead to exceptional longevity. Thus, the genotypes associated with longevity identified through comparing centenarians and middle-age controls may serve as candidates for further studies on effects of gene-environment interactions on longevity and health to determine whether they are found in other populations and cohorts that have been subjected to similar or different environmental factors.

We performed GWAS analysis using logistic regression as implemented in PLINK (1.06)¹⁶. To minimize the effects of population stratification, we adjusted for the top two eigenvectors, which corrected nearly all of the stratification that can be corrected⁵⁷. In the combined data analysis, we also adjusted for the sex and geographic stratification of Southern and Northern regions. In addition, we performed the meta-analysis, treating the two independent datasets from Southern and Northern regions of China as two groups and employing the METAL software⁶⁵.

For the SNPs associated with longevity and replicated in the datasets of the Southern and Northern regions, we also conducted gender-specific analyses and tested whether the differences in the effect sizes measured by the odds ratio were statistically significant between the two genders. We used Woolf’s test⁶⁶ to calculate *P* values to measure the gender differences in association with longevity (Supplementary Table 2). Woolf’s test is a method for testing the heterogeneity of 2×2 contingency tables over multiple strata, which is sensitive when the odds ratios (OR) of two genders are in opposite directions (the allele is protective for one sex and risky for the other), or in the same direction but of significantly different magnitude of effect size.

The methods of statistical analyses using the GWAS datasets from EU longevity genetics consortium and NECS were described in the refs 4 and 10, respectively^{4,10}.

M7. Pathway analysis. High-throughput single-SNP association analysis per se does not directly produce biologically functional findings because SNPs and genes work in an intricate network of interactions through biological pathways. In addition, each of the significant SNPs normally has a small effect on a complex trait such as longevity. Thus, pathway analysis is an important follow-up for GWAS studies to provide mechanistic insight about the underlying biology of the phenotype of interest. By identifying well-annotated pathways that map to the lists of significant genes identified in a GWAS, biochemical hypotheses can be enumerated and tested⁶⁷. Thus, to further investigate the large number of SNPs produced by our GWAS in the context of biological processes, we conducted pathway and network analysis. We applied an algorithm known as improved gene set enrichment analysis for GWAS (i-GSEA4GWAS) to place variants associated with longevity within curated pathways and functional categories⁶⁸. SNPs with $p < 0.05$ in our combined GWAS dataset were mapped to genes if within a

20 kb distance (upstream or downstream). For a given SNP, if multiple genes were located within this range, the closest gene was selected and assigned the association P value. Since multiple SNPs can map to the same gene, a SNP label permutation was used to reduce biases caused by larger loci having a disproportionately higher number of SNPs. We did not mask the non-MHC/xMHC region and set the gene size to minimum 10 and maximum 200, based on the filter criteria recommended by Ramanan *et al.*⁶⁹ The program uses the list of SNP-mapped genes to filter the collection of pathways/gene sets to obtain candidate pathways/gene sets. In total, 127,092 SNPs associated with longevity with a $p < 0.05$ were used and they were mapped to 8,085 genes. These were limited to curated pathways derived from multiple resources such as KEGG⁷⁰, REACTOME⁷¹ and BioCarta (<http://www.biocarta.com/>), and functional annotations extracted from the Gene Ontology database⁷². A modified version of the GSEA procedure was performed, adjusted for multiple testing using false discovery rate (FDR), and pathways/gene sets with corrected p -value < 0.05 and FDR < 0.05 regarded as associated with longevity traits⁶⁸.

M8. Gene interaction network analysis. To investigate whether and how the pathways may interact to affect longevity, we performed gene interaction network analysis. We first sorted the genes which were highly-represented across the 25 enriched pathways. Then we selected 35 genes which were constituents of 2 or more enriched pathways, and used the human protein-chemical interactions database STITCH 4.0⁷³ to investigate the interaction network among significant genes and among genes and chemicals. The STITCH database accumulates data from multiple sources and contains a confidence value for each interaction. The confidence scores range between 0 and 1, with a default cutoff value of 0.15. To ensure that the results of our gene interaction network analysis attain a high confidence level, we generated evaluation datasets for STITCH in which we required a confidence score of at least 0.4 (median value)⁷³.

M9. Expression Quantitative Trait Loci (eQTL) analysis. We examined the potential effects of the SNPs, identified as associated with longevity at a suggestive significance level in our GWAS, on gene expression, using the three publicly available expression Quantitative Trait Loci (eQTL) databases: (1) the Genotype-Tissue Expression (GTEx) data derived from multiple cell and tissue types (lymphoblastoid cell lines, brain tissue and human fibroblasts)⁷⁴; (2) SeeQTL database from the University of North Carolina at Chapel Hill⁷⁵; and (3) eQTL resources from the Gilad/Pritchard group at University of Chicago (<http://eqtl.uchicago.edu/Home.html>). In total, 127,092 SNPs with $P < 0.05$ in the combined datasets of our GWAS were included to examine their effects on gene expression in using these three eQTL database resources.

M10. Statements of Approval for Human Subjects Informed Consent and in accordance with relevant guidelines. The Research Ethics Committees of Duke University and Peking University granted approval for the Protection of Human Subjects for the Chinese Longitudinal Healthy Longevity Survey, including collection of DNA sample used for present study. The survey respondents who contributed their DNA samples gave informed consent before participation. All of the GWAS experiments and methods of analyses in present study were performed in accordance with relevant guidelines and regulations.

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Acknowledgements

This study is supported by the National Key Basic Research Program of China (2013CB530700) (XLT, TN, YZ), National Natural Science Foundation of China (71233001, 71490732) (YZ), NIA/NIH (R01 AG023627, YZ; Pepper Center Grant P30AG028716, ERH; U19AG023122, TP, PS). The EU GWAS on longevity was performed by the EU Longevity Genetics Consortium undertaken within the framework of European Union's Seventh Framework Programme (FP7/2007–2011) under grant agreement no 259679 (IDEAL). We are grateful to Xiaomin Shi, Zhaoxue Yin, Jieshi Luo and Yuebin Lv from China Center for Diseases Control and Prevention; Yuzhi Liu, Chunyuan Zhang, Yun Zhou and Zhenzhen Zheng from Peking University; Zhenyu Xiao, Liqun Tao, Qin Xu and Ye Yuan from Chinese Center for Aging Science Research; and Jie Zhan from China Social Science Academy for their contributions to the Chinese Longitudinal Healthy Longevity Survey (CLHLS) and the DNA sample collections, and we thank all interviewees and their families for their voluntary participation in the CLHLS study. We thank Xiaopan Liu, Qiuyan Liao, Jingzhi Chen, Hongcheng Zhou and Mingrui Li from China National GeneBank-Shenzhen for their contributions of sample storage, DNA extractions and genotyping. We are grateful for the thoughtful comments provided by Jessica Sautter. We are grateful for the following scholars from EU Longevity Genetics Consortium (including GEHA project consortium) for their contributions to the EU longevity GWAS database which was used for the look-up/comparative analysis across Han Chinese, European and U.S. ethnicities in this article: Erik B. van den Akker, Maris Alver, Karen Andersen-Ranberg, Kristin L. Ayers, Marian Beekman, Anna M. Bennet, Vladyslav Bezrukov, H len  Blanch , Stefan B hringer, Dorret I. Boomsma, Marie E. Breen, Ruud van der Breggen, Linda Broer, Amke Caliebe, Miriam Capri, Elisa Cevenini, Joanna C. Collerton, Heather J. Cordell, Anton J.M. de Craen, Paolina Crocco, Serena Dato, Karen Davies, Luca Deiana, Cornelia M. van Duijn, Krista Fischer, Friederike Flachsbart, Ian Ford, Pilar Galan, Jutta Gampe, Paolo Garagnani, Eco J.C. de Geus, Efsthatios Gonos, Daniel F. Gudbjartsson, Jennifer Harrow, Diana van Heemst, Bastiaan T. Heijmans, Femke-Anouska Heinsen, Quinta Helmer, Antti Hervonen, Albert Hofman, Jouke-Jan Hottenga, Jeanine J. Houwing-Duistermaat, Bernard Jeune, Palmi V. Jonsson, J.Wouter Jukema, Yoichiro Kamatani, Thomas B.L. Kirkwood, Peter Kristensen, Mark Lathrop, Doris Lechner, Alberta Leon, Patrik Magnusson, Carmen Martin-Ruiz, Susan E. Mcnerlan, Andres Metspalu, Evelin Mihailov, Alberto Montesanto, Simon P. Mooijaart, Anne Murphy, Almut Nebel, Christopher P. Nelson, Ellen A. Nohr, Giuseppe Passarino, Lavinia Paternoster, Pier Giuseppe Pelicci, Markus Perola, Iris Postmus, Michel Poulain, Irene Maeve Rea, Jos  Remacle, Fernando Rivadeneira, Jean Marie Robine, Giuseppina Rose, Owen A. Ross, Stefano Salvioli, Nilesh J. Samani, Naveed Sattar, Gary I. Saunders, Stefan Schreiber, Ewa Sikora, Thorkild I.A. S rensen, Liana Spazzafumo, Hreinn Stef nsson, K ri Stef nsson, David J. Stott, Riin Tamm, Henning Tiemeier, Olivier Toussaint, Stella Trompet, Hae-Won Uh, Andr  G. Uitterlinden, Alexander Viktorin, Rudi G.J. Westendorp, Gonneke Willemsen.

Author Contributions

Y.Z., H.Y., X.T., J.W.V., C.N. and E.H. designed the study. Y.Z., J.W.V., J.L. and J.X.L. designed and supervised CLHLS which recruited the participants. C.N., X.L., M.W., Y.F.Z., Y.W., L.Z., Y.W. and Z.L. contributed to sample management and performed the genotyping. C.N., X.L., H.C., H.X., Y.Q., X.L., H.C., X.X., P.S. and Y.F.Z. performed bioinformatics quality controls and the statistical analysis. W.T., Y.L., H.Y. J.Z., C.S., L.C., H.W., J.D., G.Z. and W.T. performed the DNA extractions. H.Y., J.Z. and C.S. performed independent double-check of the main parts of the GWAS data analysis. Y.Z., C.N., J.M. and X.L. wrote the initial draft, with critical revisions by J.W.V., X.T., H.Y., E.H., M.L., J.M., Q.T., E.S., J.D., P.S., T.P., H.B., C.F., K.L., J.G., W.G., J.W., J.U.W., M.N., L.C. and Z.Y. All authors revised and gave final approval of this manuscript.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Zeng, Y. *et al.* Novel loci and pathways significantly associated with longevity. *Sci. Rep.* **6**, 21243; doi: 10.1038/srep21243 (2016).



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