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Original Article

Exome-wide Association Study Identifies *CLEC3B* Missense Variant p.S106G as Being Associated With Extreme Longevity in East Asian Populations

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Abstract

Life span is a complex trait regulated by multiple genetic and environmental factors; however, the genetic determinants of extreme longevity have been largely unknown. To identify the functional coding variants associated with extreme longevity, we performed an exome-wide association study (EWAS) on a Japanese population by using an Illumina HumanExome Beadchip and a focused replication study on a Chinese population. The EWAS on two independent Japanese cohorts consisting of 530 nonagenarians/centenarians demonstrated that the G allele of *CLEC3B* missense variant p.S106G was associated with extreme longevity at the exome-wide level of significance ($p = 2.33 \times 10^{-7}$, odds ratio [OR] = 1.50). The *CLEC3B* gene encodes tetranectin, a protein implicated in the mineralization process in osteogenesis as well as in the prognosis and metastasis of cancer. The replication study consisting of 448 Chinese nonagenarians/centenarians showed that the G allele of *CLEC3B* p.S106G was also associated with extreme longevity ($p = .027$, OR = 1.51), and the p value of this variant reached 1.87×10^{-8} in the meta-analysis of Japanese and Chinese populations. In conclusion, the present study identified the *CLEC3B* p.S106G as a novel longevity-associated variant, raising the novel hypothesis that tetranectin, encoded by *CLEC3B*, plays a role in human longevity and aging.

Keywords: Longevity—Centenarian—Human aging—Human genetics

Life span is a complex trait regulated by multiple genetic and environmental factors. The higher mean life expectancy in the industrialized countries than in the developing countries indicates that environmental factors such as medicine, nutrition, and social welfare impact longevity (1). Nevertheless, twin studies have demonstrated the heritability of longevity to be 20%–30% (2–4). Furthermore, the larger genetic than environmental influence on life span has been observed in exceptionally long-lived families than in normal populations (5,6), suggesting the existence of genetic variants associated with extreme longevity. Identification of longevity-associated genes may help us to understand the mechanism of longevity and aging and also contribute to the discovery of target molecules for prevention of age-related diseases.

Up to date, a number of theories on aging such as insulin signaling (7) and free radical (oxidative stress/mitochondrial) ones (8,9) have been proposed. Researchers therefore have explored longevity-associated genes under the hypothesis that the polymorphisms in the genes associated with these aging theories are determinants of human life span (9–15). However, most single-nucleotide polymorphisms (SNPs) have not been shown to be associated with extreme longevity across populations, except for apolipoprotein E (*APOE*) (16–22) and forkhead box protein O3 (*FOXO3*) (16,23,24), which have been implicated in Alzheimer's disease onset (25,26) and insulin signaling, respectively (27).

Researchers also have explored genetic variants associated with extreme longevity by performing a genome-wide association study (GWAS), which is a powerful approach to identify the genetic loci responsible for complex traits (16–18,28). However, only the *APOE* locus was associated with longevity at the genome-wide significance level ($p < 5.0 \times 10^{-8}$) in multiple GWASs. Thus, the genetic components explaining the heritability of longevity have been largely unknown, and genetic variants that have not been uncovered by classical GWASs may underlie the heritability of longevity.

Rapid advances in DNA sequencing technology have enabled us to determine individual whole-genome sequences and have prompted researchers to explore the functional variants associated with complex traits, including low-frequency and rare variants in the protein-coding region (29). Several whole-genome sequencing studies on super-centenarians (age ≥ 110 years) have been performed to identify extreme longevity-associated coding variants; however, these studies have found no significant association because of the limited sample size (30,31). Although the sequence-based association study is currently difficult due to the high cost incurred to analyze a large number of samples, the genotyping array-based approach can resolve the cost problem and enables us to detect functional coding variants associated with extreme longevity with increased statistical power.

Here, we performed an exome-wide association study (EWAS) on extreme longevity in Japanese centenarians (age ≥ 100 years) and consecutive autopsy cases including nonagenarians/centenarians (age ≥ 95 years) by using the Infinium HumanExome BeadChip, followed by a replication study in Chinese nonagenarians/centenarians (age ≥ 95 years). By this means, we identified the association of the p.S106G missense variant of the C-type lectin domain family 3, member B (*CLEC3B*) gene encoding tetranectin with extreme longevity, thus providing a novel insight into the genetic basis of human longevity and aging.

Methods

Ethics Statement

Written informed consent was obtained from all participants or the bereaved family of each of the patients prior to the autopsy examination. The study was approved by the Ethical Committee of Keio University, National Institute for Longevity Sciences, Tokyo Metropolitan Institute of Gerontology, and Kunming Institute of Zoology, Chinese Academy of Sciences.

Study Populations

The discovery EWAS consisted of two independent Japanese cohorts. Cohort 1 consisted of 438 centenarians (mean age at death, 104.3 years; age range, 100–107 years; men, $n = 84$; women, $n = 354$) and 3,674 controls (mean age, 61.9 years; age range, 40–79 years; men, $n = 1{,}741$; women, $n = 1{,}933$). Of these centenarians, 404 were participants in the Tokyo Centenarian Study (32), who had been enrolled mainly in the Tokyo metropolis. The remaining 34 centenarians had been enrolled in Gifu Prefecture, located in the central area of Honshu, the main island of Japan (14). The control group in Cohort 1 consisted of the participants of two longitudinal studies. Of these controls, 2,903 were middle-aged and elderly people who participated in the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA) and lived in the city of Obu or in the town of Higashiura in Aichi Prefecture, located in the central area of Honshu (33). The remaining 771 controls were elderly people in metropolitan Tokyo who participated in the Itabashi Cohort Study 2011 (ITABASHI11) (34).

Cohort 2 consisted of 2,337 consecutive cases of autopsy performed at the Tokyo Metropolitan Geriatric Hospital from 1995 to 2012 (mean age at death, 80.6 years; age range, 33–104 years; men, $n = 1{,}296$; women, $n = 1{,}041$). The participants had been enrolled in the Internet database of Japanese SNPs for geriatric research (JG-SNP) (35). Because only 16 participants lived to be 100 years old in this cohort, we set the criteria of case (extreme longevity) participants at 95 years of age ($n = 109$; mean age at death, 97.3 years; age range, 95–104 years; men, $n = 33$; women, $n = 76$). Because individuals who survived for more than 80 years likely had a chance to possess longevity-associated alleles, we removed 1,194 individuals aged 80–94 years, and the data on remaining 1,034 participants were used in the case–control study (mean age at death, 72.8 years; age range, 33–79 years; men, $n = 666$; women, $n = 368$).

The replication study (Cohort 3) was performed on a Chinese cohort consisting of 447 nonagenarians/centenarians (mean age, 101.8 years; age range, 95–110 years; men, $n = 43$; women, $n = 404$) and 505 younger controls (mean age 37.9 years; age range, 15–79 years; men, $n = 256$; women, $n = 249$). All participants had been recruited from Hainan Province of China in 2010, and none of them were reported, following medical examination, to have had any severe disease when they were recruited (36).

Characteristics of each cohort and a histogram of age distribution of control samples (Cohorts 1 and 3) or age at the time of death of autopsy cases (Cohort 2, including individuals who lived to be 80–94 years) are summarized in Table 1 and Supplementary Figures 1–3.

Genotyping and Quality Control

Total DNA was extracted from peripheral blood (Cohorts 1 and 3) or kidney (Cohort 2) by using standard protocols such as phenol–chloroform extraction and spin column ones. DNA quality was evaluated by performing agarose gel electrophoresis and spectrophotometry. Genotyping of SNPs in Japanese populations was conducted with Infinium HumanExome BeadChip version 1.1 (Cohort 2) or version 1.2 (Cohort 1; Illumina, San Diego, CA) according to the manufacturer's protocol. Genotype calling was performed

Table 1. Characteristics of the Study Cohort

	Case/Control	Genotyping Platform	Number	Number of Men/Women	Current Age/Age at Death (range)
Discovery cohort (Japanese)					
Cohort 1	Case	Infinium HumanExome BeadChip ver.1.2	438	84/354	104.3 (100–107)
	Control	Infinium HumanExome BeadChip ver.1.2	3,674	1,741/1,933	61.9 (40–79)
Cohort 2	Case	Infinium HumanExome BeadChip ver.1.1	109	33/76	97.3 (95–104)
	Control	Infinium HumanExome BeadChip ver.1.1	1,034	666/368	72.8 (33–79)
Replication cohort (Chinese)					
Cohort 3	Case	Sanger sequencing	447	43/404	101.8 (95–110)
	Control	Sanger sequencing	505	256/249	37.9 (15–79)

Note: ITABASHI11, Itabashi Cohort Study 2011; NILS-LSA, National Institute for Longevity Sciences Longitudinal Study of Aging.

by using the GenTrain clustering algorithm (version 1.0) in the GenomeStudio (version. 2011.1; Illumina). Cluster boundaries were determined by use of the standard cluster files provided by Illumina. A total of 242,901 (version 1.1) or 244,770 (version 1.2) variants were genotyped and subsequently subjected to quality control. SNP genotyping in Cohort 3 was performed by use of the standard Sanger sequencing protocol. The *CLEC3B* sequences were amplified using a 5′ primer of sequence 5′-CTGGGCGGTTGGCTCTTTG-3′ and a 3′ primer 5′-AGTGTTAGTTTCCTGGCTTTGGT-3′, following the temperature profile of 94°C, 30 seconds; 64°C, 30 seconds; and 72°C, 90 seconds for 35 cycles, then ending with incubation at 72°C for 7 minutes. Polymerase chain reaction products were purified by using Exo-SAP-IT reagent before being subjected to sequencing reactions using a BigDye Terminator kit (Applied Biosystems, Carlsbad, CA). Capillary separation was performed on the ABI 3730xl Genetic Analyzer (Applied Biosystems). Sequences were aligned and edited by use of LaserGene v7.1 software (DNAStar, Madison, WI)

Quality control in the Japanese discovery cohorts was performed according to the following procedure: We first removed the participants with call rates less than 0.98 (157 samples in Cohort 1; 0 samples in Cohort 2) and subsequently excluded the SNPs with call rates less than 0.99 (7,415 SNPs in Cohort 1; 6,919 SNPs in Cohort 2), SNPs with minor allele frequency (MAF) less than 0.001 (200,709 SNPs in Cohort 1; 200,388 SNPs in Cohort 2), SNPs with deviation from the Hardy–Weinberg equilibrium ($p < 1 \times 10^{-5}$) in controls (615 SNPs in Cohort 1; 581 SNPs in Cohort 2), and SNPs in sex chromosomes and mitochondrial genome (872 SNPs in Cohort 1; 856 SNPs in Cohort 2). We also excluded the SNPs showing different call rates between cases and controls by using PLINK—test-missing option (exclusion criteria: $p < .001$ by chi-squared test; 34 SNPs in Cohort 1; 0 SNPs in Cohort 2). Duplicated samples and relatives were checked based on identity-by-descent (IBD) estimates. We calculated the IBD estimate by adopting the PLINK—genome option (37), and each pair with IBD estimate greater than 0.375 was defined as first-degree relative or duplicated sample, and either one of each pair was removed (114 samples in Cohort 1; 0 samples in Cohort 2). Outliers in the population were also checked based on pairwise identity-by-state (IBS) distance by using the PLINK—neighbor option. The IBS distance between each of five nearest neighbors was transformed into a *Z* score, with which individuals less than –4 were defined as outliers and excluded (27 samples in Cohort 1; 8 samples in Cohort 2). Both the IBD estimate and IBS distance were calculated based on the subset of modest and high linkage disequilibrium (LD) region-pruned autosomal common SNPs with MAF greater than 0.05. We used the PLINK—indep-pairwise option to exclude SNPs in LD with $r^2 \geq .2$. A window size was set at 50 SNPs, and the number of SNPs to shift the window at each step was 5. Then, 15,271 LD-pruned (Cohort 1) or 15,206 (Cohort 2) SNPs were used to calculate the IBD estimate and IBS distance. We also removed individuals with high rates of autosomal heterozygosity (> or < 3 *SD*), 82 samples in Cohort 1; 25 samples in Cohort 2). After quality control, 41,213 SNPs and 3,732 individuals consisting of 423 centenarians and 3,309 controls in Cohort 1, and 39,305 SNPs and 1,100 individuals including 107 nonagenarians/centenarians in Cohort 2 were available for analysis. We restricted the analysis to missense, stop gain, stop loss, frameshift, and splice site SNPs; and 25,089 (Cohort 1) or 23,915 (Cohort 2) of these SNPs were finally analyzed by using the single-variant association test (Figure 1). An average genotyping call rate of 99.9% for the remaining samples was achieved in both Cohorts 1 and 2.

Variant Annotation

Variant annotation was performed by using SNPnexus based on the RefSeq database (human genome build GRCh37; (38)). The variants were functionally annotated as missense, stop gain, stop loss, frameshift, and splice site.

Statistical Analysis

We performed principal component analyses (PCA) with EIGENSTRAT version 6.0.1 to account for potential population stratification in the statistical analysis for discovery EWAS (39). For PCA, 20,234 LD region-pruned autosomal (Cohort 1) or 20,114 (Cohort 2) SNPs with MAF greater than 0.01 were used; and the first 10 principal components (PCs) in each cohort were computed. Population structures of Cohorts 1 and 2 identified by PCA are shown in Supplementary Figures 4 and 5, respectively. Population stratification was evaluated by calculating genomic inflation factor λ from uncorrected or PC-corrected Cochran–Armitage trend χ^2 statistics by using EIGENSTRAT.

Single-variant associations with longevity case–control status were assessed with PLINK version 1.07 (37). In the discovery EWAS, we used logistic regression analyses adjusted for sex and the first 10 (Cohort 1) or 6 (Cohort 2) PCs, assuming additive, dominant, and recessive genetic models. A logistic regression analysis adjusted for sex was also used for the replication study on Cohort 3. We also performed a gene-based burden test to examine the association between the set of only rare and low-frequency variants with MAF less than

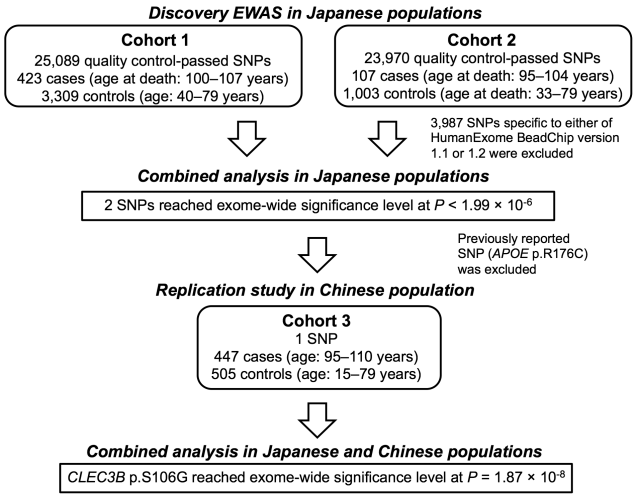

Figure 1. Flow diagram of the study.

0.05 and extreme longevity. We tested 3,374 (Cohort 1) or 3,132 (Cohort 2) genes including at least two such variants by use of the sequence kernel association optimal test (SKAT-O) (40). Gene-based association analyses were adjusted for sex and the first 10 or 6 PCs as well as for single-variant association. We set the exome-wide significance level at p less than 1.99×10^{-6} for the single-variant association and p less than 1.48×10^{-5} for the gene-based burden test according to the number of tested SNPs or genes in Cohort 1. The statistical power of the single-variant test was estimated by using the CaTS power calculator (http://www.sph.umich.edu/csg/abecasis/CaTS/index.html).

Meta-analysis of the results from single-variant association analyses in Cohorts 1–3 was performed with METAL (41). A total of 22,536 SNPs commonly included in Infinium HumanExome BeadChip version 1.1 and 1.2 were analyzed in the combined analysis of Japanese Cohorts 1 and 2. The combined p values were calculated by using the inverse-variance method from the beta coefficient and standard error obtained in each cohort. Heterogeneity of allelic effects between the discovery and replication cohorts was assessed with Cochran's Q statistics using METAL. Regional association results from combined analysis of Japanese populations were plotted by using LocusZoom (42). We also examined the sex-specific allelic effects by performing the Cochran–Armitage trend test; then the heterogeneity between men and women was assessed by use of the above-described meta-analysis procedures. Meta-analysis of the results from the SKAT-O test was carried out by use of MetaSKAT (43).

To verify that the SNPs identified as being associated with extreme longevity were also associated with overall life span, we performed Kaplan–Meier survival analysis of 2,274 quality control–passed consecutive autopsy cases including the individuals who lived to be 80–94 years by using the R package "survival" (http://cran.r-project.org/web/packages/survival/). Difference in survival curves between genotypes was compared by use of the log-rank test, assuming genotypic (two degrees of freedom), dominant, and recessive genetic models.

To identify the gene sets/pathways associated with extreme longevity, we performed a gene set enrichment analysis (GSEA) with MAGENTA version 2.4 (44) using the p values of 22,536 SNPs from combined analysis of Japanese Cohorts 1 and 2. Briefly, MAGENTA creates a gene association score from the smallest p value of the set of SNPs assigned to the genes, which are adjusted for confounders such as gene size, density of SNPs per gene, and LD. Subsequently, MAGENTA tests whether any of the gene sets are enriched for highly ranked gene association scores that are above a predefined cutoff value, and the p values for GSEA are generated by 10,000–1,000,000 permutations of gene sets. We tested 3,216 gene sets/pathways annotated from Gene Ontology, PANTHER, Ingenuity, KEGG, REACTOME, and BIOCARTA databases. We adopted the 95th percentile of the gene association scores as the cutoff and set the significance level at p less than .05 and the false-discovery rate (FDR) at less than 0.2.

Confirmation of MAF in the General Population

To confirm the MAF of EWAS-identified variants in the general population, we utilized the SNP data of individuals who had participated in the Inabe Health and Longevity Study and lived in Mie Prefecture, located in the central area of Honshu (45). SNP data on 4,499 individuals (mean age, 62.0 years; age range, 17–79 years; men, n = 1,429; women, n = 3,070) genotyped with Infinium HumanExome BeadChip version 1.2 were available for confirmation.

Results

An EWAS on extreme longevity was conducted on quality control–passed individuals of two Japanese cohorts. Genomic inflation factor λ based on the median Cochran–Armitage trend χ^2 statistics showed slight population stratification (λ = 1.105 in Cohort 1; λ = 1.093 in Cohort 2; Supplementary Figure 6A and 6C); therefore, we performed PCA to correct for potential population stratification. Genomic inflation factor λ was minimalized when χ^2 statistics were corrected by the first 10 PCs in Cohort 1 (λ = 1.046; Supplementary Figure 6B) or 6 PCs in Cohort 2 (λ = 1.068; Supplementary Figure 6D); and further correction using the genomic control method was not performed. For the single-variant association test, we used logistic regression analyses adjusted for sex and the first 10 PCs (Cohort 1) or 6 PCs (Cohort 2), assuming additive, dominant, and recessive genetic models. None of the SNPs reached the exome-wide significance ($p < 1.99 \times 10^{-6}$) in Cohort 1 or Cohort 2 alone in all of the genetic models (Supplementary Tables 1–3).

We next performed meta-analysis of the results from Cohorts 1 and 2 consisting of 530 nonagenarians/centenarians and 4,312 controls. The combined analysis of Japanese populations identified that *CLEC3B* p.Sl06G was associated with extreme longevity at the exome-wide significance level in the additive model (odds ratio [OR] = 1.50, $p = 2.33 \times 10^{-7}$; Figure 2A and Table 2). The statistical power to detect an OR of 1.50 for the G allele of *CLEC3B* p.Sl06G was 82% with $\alpha = 1.99 \times 10^{-6}$ when the survival rate of individuals at 95 years of age was postulated to be 16% (average of men and women) according to a statistical survey conducted by the Ministry of Health, Labor, and Welfare in Japan (http://www.mhlw.go.jp). *CLEC3B* p.S106G was associated with extreme longevity with comparable effect size between Cohorts 1 and 2, and the results of the Cochran Q test indicated no significant heterogeneity of the allelic effect of *CLEC3B* p.S106G between these cohorts (p_{het} = .416). The regional plot of *CLEC3B* locus is shown in Supplementary Figure 7, which clearly demonstrated that *CLEC3B* p.S106G was the lead SNP in this locus. In the combined analysis of the Japanese populations, *APOE* p.R176C, which defines the *APOE* ε2 haplotype previously reported to be associated with Alzheimer's disease and longevity (20,22,26), also reached the exome-wide significance level

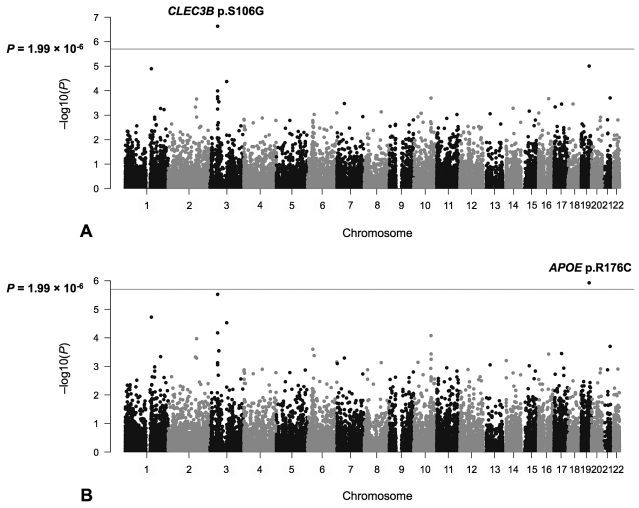

Figure 2. Manhattan plot of the *p* values from the combined analysis of Cohorts 1 and 2. (**A**) Additive genetic model. (**B**) Dominant genetic model.

in the dominant model (OR = 1.98, $p = 1.18 \times 10^{-6}$; Figure 2B and Table 2). The statistical power to detect an OR of 1.98 for the GG or AG genotype of *APOE* p.R176C was 72% with $\alpha = 1.99 \times 10^{-6}$. No cohort-specific allelic effects of *APOE* p.R176C were observed ($p_{het} = .694$). Moreover, no SNPs except for *CLEC3B* p.S106G and *APOE* p.R176C reached exome-wide significance in the combined analysis of the Japanese populations.

Our EWAS in Japanese cohorts demonstrated that *CLEC3B* p.S106G and *APOE* p.R176C were associated with extreme longevity at the exome-wide significance level in the Japanese population. Because the significant association of *APOE* p.R176C with longevity was previously reported in multiple studies by the candidate gene approach (20,22), we therefore sequenced only *CLEC3B* p.S106G in the Chinese population and performed a replication study to elucidate whether the variant was associated with longevity across east Asian populations. The replication study showed that the minor G allele (glycine 106) of *CLEC3B* p.S106G was more frequent in the Chinese nonagenarians/centenarians than in the younger controls with comparable size effect to that in the Japanese population ($p = .027$, OR = 1.51). The *p* value of the association with *CLEC3B* p.S106G reached the exome-wide significance level ($p < 1.81 \times 10^{-8}$, OR = 1.50) in the meta-analysis of Japanese and Chinese populations.

We next examined the sex-specific effects of EWAS-identified *CLEC3B* p.S106G on extreme longevity (Table 3). Although *CLEC3B* p.S106G was associated with extreme longevity with comparable OR between men and women in Cohort 1, no significant association ($p < .05$) was observed in men from Cohorts 2 and 3. However, meta-analysis of Cohorts 1–3 showed no significant heterogeneity of the allelic effect between men and women ($p_{het} = .463$), suggesting no sex-specific allelic effects of *CLEC3B* p.S106G on extreme longevity.

The MAF of *CLEC3B* p.S106G was 0.264 in the cases and 0.193 in the controls in the combined analysis of the Japanese populations. Because the MAF of *CLEC3B* p.S106G in HapMap JPT seemed to be higher than that in the control individuals in our study (MAF = 0.221, $n = 226$, http://hapmap.ncbi.nlm.nih.gov), we confirmed the MAF of this SNP in another Japanese population. The MAF of *CLEC3B* p.S106G in the individuals participating in Inabe Health and Longevity Study was 0.200. Therefore, the MAF of the *CLEC3B* p.S106G in the control samples of Japanese cohorts did not deviate from that in the general Japanese population. On the other hand, the MAF of *CLEC3B* p.S106G in the Chinese population of the present study was lower than that of the Japanese population (0.103 in the cases and 0.066 in the controls, respectively). We confirmed that the MAF of *CLEC3B* p.S106G in HapMap CHB was almost the same as that of the control participants in Cohort 3 (MAF = 0.073, $n = 82$, http://hapmap.ncbi.nlm.nih.gov).

Although we revealed that *CLEC3B* p.S106G was associated with extreme longevity defined as survival to 95 years of age in the Japanese population, it remained unknown whether this SNP would influence the overall life span. We therefore performed survival analysis of consecutive autopsy cases in Cohort 2. The *CLEC3B* p.S106G genotype was associated with age at the time of death in 2,274 consecutive autopsy cases including individuals who lived to be 80–94 years in any of genetic model ($p_{genotypic} = .025$; $p_{dominant} = .020$; $p_{recessive} = .047$, Supplementary Figure 8). However, when we analyzed the data from 2,168 individuals who lived to be less than 95 years of age, no association between the *CLEC3B* p.S106G genotype and age at the time of death was observed in any genetic model ($p_{genotypic} = .731$; $p_{dominant} = .510$; $p_{recessive} = .551$).

The single-variant association test of rare and low-frequency functional variants has insufficient statistical power even in the sample size of the present study. We therefore performed a gene-based burden test using the SKAT-O test to identify the set of rare and low-frequency variants with MAF less than 0.05 associated with extreme longevity in the Japanese population. However, none of the genes reached the exome-wide significance level in either Cohort 1 or 2 alone, and meta-analysis using MetaSKAT also did not identify the genes associated with extreme longevity (Supplementary Table 4).

Finally, we performed a GSEA to identify the gene sets/pathways associated with extreme longevity. A GSEA using MAGENTA identified 14 gene sets/pathways possibly associated with extreme longevity ($p < .05$ and FDR < 0.2; Table 4).

Discussion

We performed the first study exploring functional coding variants associated with extreme longevity by use of an exome array. Our EWAS on Japanese nonagenarians/centenarians from two independent cohorts and the replication study on Chinese nonagenarians/centenarians identified *CLEC3B* missense variant p.S106G as being associated with extreme longevity in east Asian populations.

The *CLEC3B* gene encodes tetranectin, which is a trimeric Ca^{2+}-binding protein belonging to the family of C-type lectins and is mainly found in plasma and extracellular matrix (46,47). Tetranectin binds to plasminogen kringle 4, to sulfated polysaccharides, and to extracellular matrix proteins, enhancing plasminogen activation in the presence of tissue plasminogen activator (46–48). Therefore, tetranectin is thought to participate in plasminogen activator–related biological processes such as tissue degradation/remodeling and extracellular proteolysis.

It has been suggested that tetranectin has a potential role in the mineralization process in osteogenesis (49). An earlier animal study demonstrated that delayed fracture healing in tetranectin-deficient mice was due to delayed soft tissue and callus formation, but not due to decreased capacity for cartilage and bone formation (50). Another animal study reported that ovariectomized mice, a postmenopausal osteoporosis model, exhibited a 50 times higher level of plasma tetranectin than sham mice, and authors suggested tetranectin to be a candidate biomarker reflecting bone metabolic turnover (51).

Table 2. Association of *CLEC3B* p.S106G With Extreme Longevity

Chromosome	Position	SNP	Gene	Amino Acid Change	Allele		Cohort	MAF		OR	95% CI	p	p_{het}
					Major	Minor		Case	Control				
3	45077123	rs13963	*CLEC3B*	Missense p.S106G	A	G	Cohort 1 (Japanese)	0.266	0.198	1.45	1.22–1.72	1.20×10^{-5}	
							Cohort 2 (Japanese)	0.257	0.177	1.70	1.21–2.39	0.002	
							All Japanese			1.50	1.28–1.74	2.33×10^{-7}	.416
							Cohort 3 (Chinese)	0.103	0.066	1.51	1.05–2.17	0.027	
							All east Asian			1.50	1.30–1.72	1.87×10^{-8}	.718

Notes: Data were analyzed by performing logistic regression analysis assuming the additive genetic model. Genotype counts for each SNP are listed in Supplementary Tables 1–3.
95% CI = 95% confidence interval; MAF = minor allele frequency; OR = odds ratio for the minor allele; p_{het} = p values for heterogeneity between the cohorts; SNP = single-nucleotide polymorphism.

Table 3. Association of *CLEC3B* p.G106S With Extreme Longevity in Men and Women

Chromosome	Position	SNP	Gene	Allele		Cohort	Men					Women					p_{het}
							MAF					MAF					
				Major	Minor		Case	Control	OR	95% CI	p	Case	Control	OR	95%CI	p	
3	45077123	rs13963	*CLEC3B*	A	G	Cohort 1 (Japanese)	0.269	0.196	1.51	1.05–2.18	0.025	0.265	0.200	1.45	1.20–1.75	1.42×10^{-4}	.837
						Cohort 2 (Japanese)	0.227	0.183	1.31	0.73–2.37	0.367	0.270	0.164	1.88	1.25–2.85	.002	.327
						All Japanese			1.45	1.07–1.98	0.018			1.51	1.28–1.80	2.03×10^{-6}	.821
						Cohort 3 (Chinese)	0.047	0.069	0.66	0.23–1.91	0.463	0.109	0.064	1.79	1.17–2.72	.006	.088
						All east Asian			1.37	1.01–1.84	0.040			1.55	1.32–1.82	6.01×10^{-8}	.463

Notes: Data were analyzed by performing Cochran–Armitage trend test.
95% CI = 95% confidence interval; MAF = minor allele frequency; OR = odds ratio for the minor allele; p_{het} = p values for heterogeneity between the cohorts; SNP = single-nucleotide polymorphism.

Table 4. Top Gene Sets/Pathways Identified by GSEA in Japanese Population

Database	Gene Set/Pathway	Effective Gene Set Size*	Expected Genes Above 95% Cutoff†	Observed Genes Above 95% Cutoff‡	*p*	FDR
PANTHER Molecular Function	Transcription factor	198	5	16	2.2×10^{-5}	.019
BIOCARTA	GATA3 pathway	16	1	4	.001	.068
Gene Ontology	Chylomicron	12	0	4	2.0×10^{-4}	.108
Gene Ontology	Antioxidant activity	9	0	3	.002	.114
Gene Ontology	Lipid homeostasis	11	0	4	3.0×10^{-4}	.126
BIOCARTA	NOS1 pathway	22	1	4	.003	.131
Gene Ontology	Associative learning	13	0	3	.003	.131
Gene Ontology	Visual learning	25	1	5	8.0×10^{-4}	.149
Gene Ontology	Negative regulation of endothelial cell proliferation	15	1	4	.002	.159
PANTHER Biological Process	Amino acid biosynthesis	46	2	7	.001	.162
Gene Ontology	Inner ear development	15	1	4	.001	.178
Gene Ontology	1-Acylglycerol-3-phosphate O-acyltransferase activity	9	0	3	.005	.181
Gene Ontology	Response to ethanol	60	2	8	5.0×10^{-4}	.186
Gene Ontology	Very-low-density lipoprotein particle	20	1	4	.002	.186

Notes: FDR = false-discovery rate; GSEA = gene set enrichment analysis; SNP = single-nucleotide polymorphism.
*The number of genes analyzed after excluding genes with no SNPs.
†The expected number of genes ranked above 95th percentile of the gene association scores.
‡The observed number of genes ranked above 95th percentile of the gene association scores.

In humans, although no association of the circulating tetranectin level with the bone phenotype has been reported, previous studies suggested that *CLEC3B* genetic variations were associated with susceptibility to osteoarthritis and osteoporosis. Valdes and colleagues genotyped *CLEC3B* p.S106G in 603 knee osteoarthritis patients and demonstrated that the major A allele (serine 106) of the *CLEC3B* p.S106G, the frequency of which was lower in the nonagenarians/centenarians in our study, was associated with an increased risk of knee osteoarthritis in both men and women (men: OR = 1.49; women: OR = 1.45; (52)). Furthermore, a linkage analysis of 1,094 pedigrees identified quantitative trait loci for bone mineral density at 3p21, which contains the *CLEC3B* gene (53). Considering that knee osteoarthritis and osteoporosis increase the risk of impaired mobility (54,55), which increases the risk of mortality (56,57), the minor G allele (glycine 106) of *CLEC3B* p.S106G may be associated with extreme longevity in part via the promotion of bone and articular health.

Tetranectin plays roles in tissue degradation and extracellular proteolysis, which are activities associated with tumor invasion (58). Therefore, it has been investigated as a potential biomarker for prognosis and metastasis of cancer. Previous studies demonstrated that a low level of circulating tetranectin is associated with a poor treatment response to anticancer drugs and survival from various types of cancer (59–61). Low levels of circulating tetranectin were also observed in patients with multiple tumors or metastases (61–63). Furthermore, the expression level of tetranectin in tumor tissue is decreased in patients with poor survival from cancer (64). These findings suggest that tetranectin is a potent inhibitor of tumor invasion and metastasis.

The present study demonstrated that *CLEC3B* p.S106G was not significantly associated with age at death in the Japanese autopsy cases that lived to be less than 95 years old, suggesting that *CLEC3B* p.S106G was only associated with whether or not people could survive until 95 years of age in the Japanese population. A statistical survey conducted by the Ministry of Health, Labor and Welfare in Japan (http://www.mhlw.go.jp) indicates that the main cause of death is quite different according to whether the individuals live to be 95 years old or not. The survey examining the cause of death in each 5-year age group showed that cancer is the leading or second cause of death in Japanese people who died at 30–94 years of age. Indeed, 28.9% of Japanese people died due to cancer. In contrast, cancer is the fifth cause of death in individuals older than 95 years; and the mortality rate from cancer is dramatically decreased to 8.0%. These statistics suggest that long-lived individuals have a genetic advantage against death from cancer, which may be due to the presence of *CLEC3B* p.S106G.

Another significant finding of the present study was that the *APOE* p.R176C missense variant was associated with extreme longevity at the exome-wide significance level in the Japanese population. *APOE* p.R176C defines the *APOE* ε2 haplotype, which is known as a protective allele against Alzheimer's disease, and several candidate gene approaches showed a higher frequency of *APOE* ε2 in long-lived individuals (20,22,26). Because *APOE* p.R176C is not covered by most conventional SNP arrays, previous GWASs on longevity only identified and replicated the SNPs in LD with *APOE* p.C130R, which defines the *APOE* ε4 haplotype (1,16,17,26). The present study demonstrated that not only *APOE* ε4 but also *APOE* ε2 was a stronger genetic determinant of extreme longevity than other genetic components except for *CLEC3B* p.S106G. Nevertheless, a previous study examining the *APOE* haplotype of centenarians in various ethnic populations showed that significant associations between *APOE* ε2 and extreme longevity were observed only in the Italian and Japanese cohorts that shared 404 centenarians with our discovery cohort (20). Replication studies on other ethnic populations are needed to elucidate whether *APOE* p.R176C is associated with life span regardless of ethnicity.

Our GSEA analysis demonstrated that 14 enriched gene sets/pathways were possibly associated with extreme longevity in the Japanese population. Interestingly, several gene sets/pathways, such as those involved in inflammation/oxidative stress (GATA3 pathway, antioxidant activity, and NOS1 pathway), lipid metabolism (chylomicron, lipid homeostasis, and very-low-density lipoprotein particle), and brain function (associative learning, visual learning, and inner ear development), have been proposed to be associated with longevity (8,65) or age-associated diseases (eg, coronary heart diseases and Alzheimer's disease) (66–68). The coding variants in these gene sets/pathways may interactively regulate the variability of the human life span even though each variant alone has a small effect on longevity. Nevertheless, our findings should be replicated in other populations to confirm that the coding variants in these gene sets/pathways determine extreme longevity.

Whole-genome and exome sequencing data from more than 1,000 individuals have revealed that numerous low-frequency and rare disease-causing variants exist in general populations (69,70), thus raising the hypothesis that long-lived individuals who are resistant to chronic diseases have fewer disease-causing variants than does the general population. However, we did not identify low-frequency and rare coding variants with MAF less than 0.05 associated with extreme longevity by either single-variant association analysis or gene-based burden testing except for *APOE* p.R176C with MAF equal to 0.048 due to the limited sample size of cases. The statistical power did not reach 80% even for the *APOE* p.R176C in the combined analysis of Cohorts 1 and 2. Association analysis using a larger sample size is required to conclude that low-frequency and rare coding variants contribute to the genetics of extreme longevity. Furthermore, because the 244,770 markers in the Infinium HumanExome BeadChip were adopted based on the whole-genome and exome sequencing data mainly obtained from individuals of European ancestry, only 25,089 functional variants were polymorphic and available for the association analysis of the Japanese population (Cohort 1). Thus, we might overlook the association of Japanese population–specific low-frequency and rare variants with extreme longevity. Today, large-scale whole-genome sequencing of 1,070 healthy individuals has identified approximately 12 million novel rare variants in the Japanese population (71), thus allowing us to explore Japanese population–specific low-frequency and rare variants associated with extreme longevity in the near future.

In conclusion, EWAS on Japanese nonagenarians/centenarians from two independent cohorts and a replication study on Chinese nonagenarians/centenarians identified the *CLEC3B* missense variant p.S106G, encoding tetranectin, as being associated with human longevity in east Asian populations. Our findings prompt us to posit the novel hypothesis that tetranectin encoded by *CLEC3B* plays a role in longevity. Further replication studies on the life span in multiethnic populations as well as functional studies on the *CLEC3B* p.S106G polymorphism should eventually elucidate the genetic basis of human longevity and aging.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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Conflict of Interest

The authors do not have any conflicts of interest to disclose.

References

1. Salomon JA, Wang H, Freeman MK, et al. Healthy life expectancy for 187 countries, 1990–2010: a systematic analysis for the Global Burden Disease Study 2010. *Lancet*. 2012;380:2144–2162. doi:10.1016/S0140-6736(12)61690-0
2. Herskind AM, McGue M, Holm NV, Sørensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. *Hum Genet*. 1996;97:319–323. doi:10.1007/BF02185763
3. Mitchell BD, Hsueh WC, King TM, et al. Heritability of life span in the Old Order Amish. *Am J Med Genet*. 2001;102:346–352. doi:10.1002/ajmg.1483
4. Skytthe A, Pedersen NL, Kaprio J, et al. Longevity studies in GenomEUtwin. *Twin Res*. 2003;6:448–454. doi:10.1375/136905203770326457
5. Schoenmaker M, de Craen AJ, de Meijer PH, et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. *Eur J Hum Genet*. 2006;14:79–84. doi:10.1038/sj.ejhg.5201508
6. Sebastiani P, Perls TT. The genetics of extreme longevity: lessons from the new England centenarian study. *Front Genet*. 2012;3:277. doi:10.3389/fgene.2012.00277
7. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science*. 2003;299:1346–1351. doi:10.1126/science.1081447
8. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956;11:298–300. doi:10.1093/geronj/11.3.298
9. Loeb LA, Wallace DC, Martin GM. The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mtDNA mutations. *Proc Natl Acad Sci USA*. 2005;102:18769–18770. doi:10.1073/pnas.0509776102
10. De Benedictis G, Rose G, Carrieri G, et al. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. *FASEB J*. 1999;13:1532–1536.
11. Debrabant B, Soerensen M, Flachsbart F, et al. Human longevity and variation in DNA damage response and repair: study of the contribution of sub-processes using competitive gene-set analysis. *Eur J Hum Genet*. 2014;22:1131–1136. doi:10.1038/ejhg.2013.299

12. Morris BJ, Donlon TA, He Q, et al. Genetic analysis of TOR complex gene variation with human longevity: a nested case-control study of American men of Japanese ancestry. *J Gerontol A Biol Sci Med Sci*. 2015;70:133–142. doi:10.1093/gerona/glu021
13. Morris BJ, Donlon TA, He Q, et al. Association analyses of insulin signaling pathway gene polymorphisms with healthy aging and longevity in Americans of Japanese ancestry. *J Gerontol A Biol Sci Med Sci*. 2014;69:270–273. doi:10.1093/gerona/glt082
14. Tanaka M, Gong JS, Zhang J, Yoneda M, Yagi K. Mitochondrial genotype associated with longevity. *Lancet*. 1998;351:185–186. doi:10.1016/S0140-6736(05)78211-8
15. van Heemst D, Beekman M, Mooijaart SP, et al. Reduced insulin/IGF-1 signalling and human longevity. *Aging Cell*. 2005;4:79–85. doi:10.1111/j.1474-9728.2005.00148.x
16. Broer L, Buchman AS, Deelen J, et al. GWAS of longevity in CHARGE consortium confirms APOE and FOXO3 candidacy. *J Gerontol A Biol Sci Med Sci*. 2015;70:110–118. doi:10.1093/gerona/glu166
17. Deelen J, Beekman M, Uh HW, et al. Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age. *Hum Mol Genet*. 2014;23:4420–4432. doi:10.1093/hmg/ddu139
18. Deelen J, Beekman M, Uh HW, et al. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. *Aging Cell*. 2011;10:686–698. doi:10.1111/j.1474-9726.2011.00705.x
19. Frisoni GB, Louhija J, Geroldi C, Trabucchi M. Longevity and the epsilon2 allele of apolipoprotein E: the Finnish Centenarians Study. *J Gerontol A Biol Sci Med Sci*. 2001;56:M75–M78. doi:10.1093/gerona/56.2.M75
20. Garatachea N, Emanuele E, Calero M, et al. ApoE gene and exceptional longevity: insights from three independent cohorts. *Exp Gerontol*. 2014;53:16–23. doi:10.1016/j.exger.2014.02.004
21. Nebel A, Kleindorp R, Caliebe A, et al. A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. *Mech Ageing Dev*. 2011;132:324–330. doi:10.1016/j.mad.2011.06.008
22. Schachter F, Faure-Delanef L, Guenot F, et al. Genetic associations with human longevity at the APOE and ACE loci. *Nat Genet*. 1994;6:29–32. doi:10.1038/ng0194-29
23. He Q, Morris BJ, Grove JS, et al. Shorter men live longer: association of height with longevity and FOXO3 genotype in American men of Japanese ancestry. *PLoS One*. 2014;9:e94385. doi:10.1371/journal.pone.0094385
24. Willcox BJ, Donlon TA, He Q, et al. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA*. 2008;105:13987–13992. doi:10.1073/pnas.0801030105
25. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993;261:921–923. doi:10.1126/science.8346443
26. West HL, Rebeck GW, Hyman BT. Frequency of the apolipoprotein E epsilon 2 allele is diminished in sporadic Alzheimer disease. *Neurosci Lett*. 1994;175:46–48. doi:10.1016/0304-3940(94)91074-X
27. Ni YG, Wang N, Cao DJ, et al. FoxO transcription factors activate Akt and attenuate insulin signaling in heart by inhibiting protein phosphatases. *Proc Natl Acad Sci USA*. 2007;104:20517–20522. doi:10.1073/pnas.0610290104
28. Newman AB, Walter S, Lunetta KL, et al. A meta-analysis of four genome-wide association studies of survival to age 90 years or older: the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium. *J Gerontol A Biol Sci Med Sci*. 2010;65:478–487. doi:10.1093/gerona/glq028
29. Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. *Cell*. 2013;155:27–38. doi:10.1016/j.cell.2013.09.006
30. Gierman HJ, Fortney K, Roach JC, et al. Whole-genome sequencing of the world's oldest people. *PLoS One*. 2014;9:e112430. doi:10.1371/journal.pone.0112430
31. Sebastiani P, Riva A, Montano M, et al. Whole genome sequences of a male and female supercentenarian, ages greater than 114 years. *Front Genet*. 2011;2:90. doi:10.3389/fgene.2011.00090
32. Gondo Y, Hirose N, Arai Y, et al. Functional status of centenarians in Tokyo, Japan: developing better phenotypes of exceptional longevity. *J Gerontol A Biol Sci Med Sci*. 2006;61:305–310. doi:10.1093/gerona/61.3.305
33. Shimokata H, Ando F, Niino N. A new comprehensive study on aging—the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). *J Epidemiol*. 2000;10(1 suppl):S1–S9. doi:10.2188/jea.10.1sup_1
34. Seino S, Shinkai S, Fujiwara Y, et al. Reference values and age and sex differences in physical performance measures for community-dwelling older Japanese: a pooled analysis of six cohort studies. *PLoS One*. 2014;9:e99487. doi:10.1371/journal.pone.0099487
35. Sawabe M, Arai T, Kasahara I, et al. Developments of geriatric autopsy database and Internet-based database of Japanese single nucleotide polymorphisms for geriatric research (JG-SNP). *Mech Ageing Dev*. 2004;125:547–552. doi:10.1016/j.mad.2004.06.005
36. He YH, Lu X, Wu H, et al. Mitochondrial DNA content contributes to healthy aging in Chinese: a study from nonagenarians and centenarians. *Neurobiol Aging*. 2014;35:e1771–1774. doi:10.1016/j.neurobiolaging.2014.01.015
37. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575. doi:10.1086/519795
38. Chelala C, Khan A, Lemoine NR. SNPnexus: a web database for functional annotation of newly discovered and public domain single nucleotide polymorphisms. *Bioinformatics*. 2009;25:655–661. doi:10.1093/bioinformatics/btn653
39. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904–909. doi:10.1038/ng1847
40. Lee S, Emond MJ, Bamshad MJ, et al. Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. *Am J Hum Genet*. 2012;91:224–237. doi:10.1016/j.ajhg.2012.06.007
41. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26:2190–2191. doi:10.1093/bioinformatics/btq340
42. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26:2336–2337. doi:10.1093/bioinformatics/btq419
43. Lee S, Teslovich TM, Boehnke M, Lin X. General framework for meta-analysis of rare variants in sequencing association studies. *Am J Hum Genet*. 2013;93:42–53. doi:10.1016/j.ajhg.2013.05.010
44. Segre AV, DIAGRAM Consortium, MAGIC investigators, et al. Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. *PLoS Genet*. 2010;6:e1001058. doi:10.1371/journal.pgen.1001058
45. Yamada Y, Matsui K, Takeuchi I, Oguri M, Fujimaki T. Association of genetic variants with hypertension in a longitudinal population-based genetic epidemiological study. *Int J Mol Med*. 2015;35:1189–1198. doi:10.3892/ijmm.2015.2151
46. Christensen L, Clemmensen I. Tetranectin immunoreactivity in normal human tissues. An immunohistochemical study of exocrine epithelia and mesenchyme. *Histochemistry*. 1989;92:29–35. doi:10.1007/BF00495012
47. Clemmensen I, Petersen LC, Kluft C. Purification and characterization of a novel, oligomeric, plasminogen kringle 4 binding protein from human plasma: tetranectin. *Eur J Biochem*. 1986;156:327–333. doi:10.1111/j.1432-1033.1986.tb09586.x
48. Kluft C, Jie AF, Los P, de Wit E, Havekes L. Functional analogy between lipoprotein(a) and plasminogen in the binding to the kringle 4 binding protein, tetranectin. *Biochem Biophys Res Commun*. 1989;161:427–433. doi:10.1016/0006-291X(89)92616-8
49. Wewer UM, Ibaraki K, Schjørring P, Durkin ME, Young MF, Albrechtsen R. A potential role for tetranectin in mineralization during osteogenesis. *J Cell Biol*. 1994;127:1767–1775. doi:10.1083/jcb.127.6.1767
50. Iba K, Abe Y, Chikenji T, Kanaya K, Chiba H, Sasaki K, Dohke T, Wada T, Yamashita T. Delayed fracture healing in tetranectin-deficient mice. *J Bone Miner Metab*. 2013;31:399–408. doi:10.1007/s00774-013-0436-y

51. Sasaki K, Ozasa Y, Iba K, et al. Significant increase of plasma tetranectin in ovx mice as defined by proteomics analysis. *J Orthop Sci*. 2014;19:809–819. doi:10.1007/s00776-014-0586-3
52. Valdes AM, Van Oene M, Hart DJ, et al. Reproducible genetic associations between candidate genes and clinical knee osteoarthritis in men and women. *Arthritis Rheum*. 2006;54:533–539. doi:10.1002/art.21621
53. Wilson SG, Reed PW, Bansal A, et al. Comparison of genome screens for two independent cohorts provides replication of suggestive linkage of bone mineral density to 3p21 and 1p36. *Am J Hum Genet*. 2003;72:144–155. doi:10.1086/345819
54. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int*. 2006;17:1726–1733. doi:10.1007/s00198-006-0172-4
55. Ling SM, Fried LP, Garrett ES, Fan MY, Rantanen T, Bathon JM. Knee osteoarthritis compromises early mobility function: The Women's Health and Aging Study II. *J Rheumatol*. 2003;30:114–120.
56. Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA. Mortality after all major types of osteoporotic fracture in men and women: an observational study. *Lancet*. 1999;353:878–882. doi:10.1016/S0140-6736(98)09075-8
57. Nüesch E, Dieppe P, Reichenbach S, Williams S, Iff S, Jüni P. All cause and disease specific mortality in patients with knee or hip osteoarthritis: population based cohort study. *BMJ*. 2011;342:d1165. doi:10.1136/bmj.d1165
58. Dano K, Behrendt N, Hoyer-Hansen G, et al. Plasminogen activation and cancer. *Thromb Haemost*. 2005;93:676–681. doi:10.1267/THRO05040676
59. Høgdall CK, Christiansen M, Nørgaard-Pedersen B, Bentzen SM, Kronborg O, Clemmensen I. Plasma tetranectin and colorectal cancer. *Eur J Cancer*. 1995;31A:888–894. doi:10.1016/0959-8049(94)00520-6
60. Høgdall CK, Hørding U, Nørgaard-Pedersen B, Toftager-Larsen K, Clemmensen I. Serum tetranectin and CA-125 used to monitor the course of treatment in ovarian cancer patients. *Eur J Obstet Gynecol Reprod Biol*. 1994;57:175–178. doi:10.1016/0028-2243(94)90296-8
61. Høgdall CK, Sölétormos G, Nielsen D, Nørgaard-Pedersen B, Dombernowsky P, Clemmensen I. Prognostic value of serum tetranectin in patients with metastatic breast cancer. *Acta Oncol*. 1993;32:631–636. doi:10.3109/02841869309092443
62. Jensen BA, Clemmensen I. Plasma tetranectin is reduced in cancer and related to metastasia. *Cancer*. 1988;62:869–872. doi:10.1002/1097-0142(19880901)62:5<869::AID-CNCR2820620503>3.0.CO;2-6
63. Nielsen H, Clemmensen I, Nielsen HJ, Drivsholm A. Decreased tetranectin in multiple myeloma. *Am J Hematol*. 1990;33:142–144. doi:10.1002/ajh.2830330213
64. Obrist P, Spizzo G, Ensinger C, et al. Aberrant tetranectin expression in human breast carcinomas as a predictor of survival. *J Clin Pathol*. 2004;57:417–421. doi:10.1136/jcp.2003.010058
65. Chung HY, Kim HJ, Kim JW, Yu BP. The inflammation hypothesis of aging: molecular modulation by calorie restriction. *Ann NY Acad Sci*. 2001;928:327–335. doi:10.1111/j.1749-6632.2001.tb05662.x
66. Castelli WP, Anderson K, Wilson PW, Levy D. Lipids and risk of coronary heart disease. The Framingham Study. *Ann Epidemiol*. 1992;2:23–28. doi:10.1016/1047-2797(92)90033-M
67. Fowler KS, Saling MM, Conway EL, Semple JM, Louis WJ. Paired associate performance in the early detection of DAT. *J Int Neuropsychol Soc*. 2002;8:58–71. doi:10.1017/S1355617701020069
68. Freedman M, Oscar-Berman M. Spatial and visual learning deficits in Alzheimer's and Parkinson's disease. *Brain Cogn*. 1989;11:114–126. doi:10.1016/0278-2626(89)90009-2
69. Genomes Project Consortium, Abecasis GR, Auton A, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491:56–65. doi:10.1038/nature11632
70. Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science*. 2012;337:64–69. doi:10.1126/science.1219240
71. Nagasaki M, Yasuda J, Katsuoka F, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. *Nat Commun*. 2015;6:8018. doi:10.1038/ncomms9018