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# Genetic landscape of *APOE* in human longevity revealed by high-throughput sequencing

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## Abstract

Apolipoprotein E (*APOE*) gene has been the most replicated longevity-associated gene in humans. Two common *APOE* alleles are either significantly depleted (ε4 allele) or enriched (ε2 allele) in long-lived individuals as compared to controls. We performed high-throughput sequencing analysis of exons and 2 kb proximal promoter of *APOE* in 450 centenarians and 500 controls of Ashkenazi Jewish decent. We found two common regulatory variants, rs405509 (p=0.006) and rs769449 (p=0.036), that were significantly depleted in centenarians. Genotyping analysis of rs7412 and rs429358 showed significant enrichment of ε2 allele (p=0.003) and ε2/ε3 genotype (p= 0.005), and significant depletion of ε3/ε4 genotype (p=0.005) in centenarians. Our findings support the hypothesis that variants in both coding and regulatory regions of *APOE* may contribute to longevity in humans.

### Keywords

APOE; Centenarian; Genetic variant; Longevity; Pooled target capture sequencing

## Description

Genome-wide association studies (GWAS) have achieved great success in identifying common genetic variants robustly associated with increased risk of complex traits and

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**Competing interests**

The authors declare that they have no competing interests.

diseases (http://www.genome.gov/gwastudies). Thus far, GWAS involving long-lived individuals have identified the chromosome 19q13.2 harboring *APOE* gene as the single most replicated longevity-associated locus, confirming the previous single gene association studies. (Beekman et al., 2013; Deelen et al., 2011; Nebel et al., 2011; Sebastiani et al., 2012). *APOE* has two common missense variants, rs429358 (Cys130Arg) and rs7412 (Arg176Cys), and a combination of the two determines functional alleles of *APOE*: ε2 (Cys130, Cys176), ε3 (Cys130, Arg176) and ε4 (Arg130, Arg176). While depletion of *APOE* ε4 allele and/or enrichment of *APOE* ε2 allele has been found to be associated with longevity, *APOE* ε4 allele has been associated with risk of multiple age-related disease such as Alzheimer's disease and cardiovascular disease (Christensen et al., 2006), and supporting the role of the common functional *APOE* variants in healthspan and lifespan.

In this study, to further discover all possible variants in coding and regulatory region of *APOE* gene, we took advantage of an efficient pooled target capture next-generation sequencing approach (Pool-seq) (Bansal et al., 2011). Our study population consist of 450 Ashkenazi Jewish centenarians (mean age: 98) older than 95 years old and 500 controls (mean age: 73) without family history of longevity. Using this population, we have successfully identified longevity associated-phenotypes, -genotypes, and their association with health outcomes (Atzmon et al., 2006; Barzilai et al., 2006; Barzilai et al., 2003). In particular, we identified two rare missense variants in the IGF-1 receptor gene (IGF1R) that were enriched in Ashkenazi Jewish centenarians as compared to younger elderly and we further showed that they were true reduced-function variants (Suh et al., 2008; Tazearslan et al., 2011). These results highlight the value of resequencing candidate genes in our Ashkenazi Jewish centenarian cohort to identify clinically relevant targets in humans. As a result of this study, we discovered 33 common and rare variants in both coding and regulatory region of *APOE* gene, 17 of them were novel variants not identified previously (Figure 1 and Supplementary Table 1). Among them, longevity-associated common variants were rs405509 (p=0.006) and rs769449 (p=0.036) in upstream and intronic region respectively, that were depleted in centenarians and may be functional based on the prediction by RegulomeDB score (http://regulomedb.org/) (Supplementary Table 1).

Our target capture sequencing performed poorly in exon 4 region of *APOE* gene harboring previously reported longevity-associated alleles (rs429358 (c.T388C:p.C130R) and rs7412 (c.C526T:p.R176C)) because of the high GC content (Supplementary Figure 1). Therefore, to validate previously reported longevity-associated *APOE* alleles, we performed genotyping of rs7412 and rs427580 using an independent Taqman (Life Technologies) SNP genotyping assay. As a result of a successful genotyping in 438 centenarians and 515 controls, rs7412 was significantly enriched (p-value: 0.001), while rs429358 was significantly depleted (p-value: 0.038) in centenarians (Table 1). Based on the genotyped variants, *APOE* ε2 (rs429328: T / rs7412: C>T), ε3 (rs429328: T / rs7412: C), and ε4 (rs429328: T>C / rs7412: C) alleles were determined. We examined the association of *APOE* alleles with longevity using allelic test of association and found that ε2 allele was significantly enriched in centenarians (9.82%) as compared to controls (6.02%) (Odd Ratio (OR): 1.70, p-value: 0.003), whereas ε4 allele was depleted in centenarians (6.62%) as compared to controls (9.03%) (OR: 0.71, p-value: 0.061) (Table 1). In addition, the genotypic test of association revealed that ε2/ε3 genotype was enriched in centenarians (16.4%) as compared to controls

(10.1%) (OR: 1.75, p-value: 0.005), conversely, ε3/ε4 genotype was significantly depleted in centenarians (9.59%) compared to controls (15.7%) (OR: 0.57, p-value: 0.005) (Table 1). These results are in concordance with previous reports that *APOE* ε2 is enriched and *APOE* ε4 is depleted in centenarians (Christensen et al., 2006). Here we validated longevity-associated *APOE* ε2 allele in our Ashkenazi Jewish population.

To investigate the effect of the *APOE* gene alleles and genotypes on phenotypes such as lipid metabolism, we tested its association with lipid profiles including serum level of cholesterol, HDL, LDL, and triglyceride. Carriers of the *APOE* ε2 allele showed significantly lower LDL levels compared to the ε3 and ε4 carriers (91.7 +/− 5.26 vs.109 +/− 1.51 mg/dL, p=0.002) (Table 1). Serum cholesterol levels were lower in ε2 allele carriers with marginal significance (p-value: 0.052) (Supplementary Table 2). On the other hand, the ε3 and ε4 alleles were not associated with lipid profile even though ε3 and ε4 allele carriers showed a trend of increased level compared to ε2 allele carriers (Table 1). In addition, LDL level was significantly lower among ε2/ε3 genotype carriers compared with the other genotype carriers (93.9 +/− 5.75 vs.109 +/− 2.19 mg/dL, p=0.01) that is concordant with the effect of ε2 allele (Table 1).

In conclusion, we discovered all possible variants in coding and regulatory region of *APOE* gene in centenarians and controls by an efficient Pool-seq in large population. Interestingly, Poolseq with target region including regulatory region such as proximal upstream and intronic region identified longevity-associated common variants, rs405509 and rs769449 that were previously associated with longevity and other aging-related disease phenotypes (Lu et al., 2014; Soerensen et al., 2013). They are also predicted (Supplementary Figure 2) and experimentally proved (Supplementary Table 3) to be functional, implicating the potential role of regulatory variants in a longevity-association. Moreover, by independent genotyping, we replicated the significant enrichment of longevity-associated *APOE* ε2 allele and ε2/ε3 genotype among our Ashkenazi Jewish cohort for the first time, and we also validated the association of *APOE* ε2 allele with beneficial low LDL level as reported previously (Ferreira et al., 2010; Volcik et al., 2006), suggesting a functional effect of the *APOE* allele and genotype that may lead to longevity. Therefore, it would be interesting to further understand a protective role of longevity-associated, predicted functional variants in *APOE* gene.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References

Atzmon G, Rincon M, Schechter CB, Shuldiner AR, Lipton RB, Bergman A, Barzilai N. Lipoprotein genotype and conserved pathway for exceptional longevity in humans. PLoS biology. 2006; 4:e113. [PubMed: 16602826]

Bansal V, Tewhey R, Leproust EM, Schork NJ. Efficient and cost effective population resequencing by pooling and in-solution hybridization. PLoS One. 2011; 6:e18353. [PubMed: 21479135]

Barzilai N, Atzmon G, Derby CA, Bauman JM, Lipton RB. A genotype of exceptional longevity is associated with preservation of cognitive function. Neurology. 2006; 67:2170–2175. [PubMed: 17190939]

Barzilai N, Atzmon G, Schechter C, Schaefer EJ, Cupples AL, Lipton R, Cheng S, Shuldiner AR. Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA. 2003; 290:2030–2040. [PubMed: 14559957]

Beekman M, Blanche H, Perola M, Hervonen A, Bezrukov V, Sikora E, Flachsbart F, Christiansen L, De Craen AJ, Kirkwood TB, Rea IM, Poulain M, Robine JM, Valensin S, Stazi MA, Passarino G, Deiana L, Gonos ES, Paternoster L, Sorensen TI, Tan Q, Helmer Q, van den Akker EB, Deelen J, Martella F, Cordell HJ, Ayers KL, Vaupel JW, Tornwall O, Johnson TE, Schreiber S, Lathrop M, Skytthe A, Westendorp RG, Christensen K, Gampe J, Nebel A, Houwing-Duistermaat JJ, Slagboom PE, Franceschi C. consortium, G. Genome-wide linkage analysis for human longevity: Genetics of Healthy Aging Study. Aging cell. 2013; 12:184–193. [PubMed: 23286790]

Christensen K, Johnson TE, Vaupel JW. The quest for genetic determinants of human longevity: challenges and insights. Nat Rev Genet. 2006; 7:436–448. [PubMed: 16708071]

Deelen J, Beekman M, Uh HW, Helmer Q, Kuningas M, Christiansen L, Kremer D, van der Breggen R, Suchiman HE, Lakenberg N, van den Akker EB, Passtoors WM, Tiemeier H, van Heemst D, de Craen AJ, Rivadeneira F, de Geus EJ, Perola M, van der Ouderaa FJ, Gunn DA, Boomsma DI, Uitterlinden AG, Christensen K, van Duijn CM, Heijmans BT, Houwing-Duistermaat JJ, Westendorp RG, Slagboom PE. 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. [PubMed: 21418511]

Ferreira CN, Carvalho MG, Fernandes AP, Lima LM, Loures-Valle AA, Dantas J, Janka Z, Palotas A, Sousa MO. Comparative study of apolipoprotein-E polymorphism and plasma lipid levels in dyslipidemic and asymptomatic subjects, and their implication in cardio/cerebro-vascular disorders. Neurochem Int. 2010; 56:177–182. [PubMed: 19819279]

Lu F, Guan H, Gong B, Liu X, Zhu R, Wang Y, Qian J, Zhou T, Lan X, Wang P, Lin Y, Ma S, Lin H, Zhu X, Chen R, Zhu X, Shi Y, Yang Z. Genetic variants in PVRL2-TOMM40-APOE region are associated with human longevity in a Han Chinese population. PLoS One. 2014; 9:e99580. [PubMed: 24924924]

Nebel A, Kleindorp R, Caliebe A, Nothnagel M, Blanche H, Junge O, Wittig M, Ellinghaus D, Flachsbart F, Wichmann HE, Meitinger T, Nikolaus S, Franke A, Krawczak M, Lathrop M, Schreiber S. A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. Mechanisms of ageing and development. 2011; 132:324–330. [PubMed: 21740922]

Sebastiani P, Solovieff N, Dewan AT, Walsh KM, Puca A, Hartley SW, Melista E, Andersen S, Dworkis DA, Wilk JB, Myers RH, Steinberg MH, Montano M, Baldwin CT, Hoh J, Perls TT. Genetic signatures of exceptional longevity in humans. PLoS One. 2012; 7:e29848. [PubMed: 22279548]

Soerensen M, Dato S, Tan Q, Thinggaard M, Kleindorp R, Beekman M, Suchiman HE, Jacobsen R, McGue M, Stevnsner T, Bohr VA, de Craen AJ, Westendorp RG, Schreiber S, Slagboom PE, Nebel A, Vaupel JW, Christensen K, Christiansen L. Evidence from case-control and longitudinal studies supports associations of genetic variation in APOE, CETP, and IL6 with human longevity. Age (Dordr). 2013; 35:487–500. [PubMed: 22234866]

Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P. Functionally significant insulin-like growth factor I receptor mutations in centenarians. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:3438–3442. [PubMed: 18316725]

Tazearslan C, Huang J, Barzilai N, Suh Y. Impaired IGF1R signaling in cells expressing longevity-associated human IGF1R alleles. Aging cell. 2011; 10:551–554. [PubMed: 21388493]

Volcik KA, Barkley RA, Hutchinson RG, Mosley TH, Heiss G, Sharrett AR, Ballantyne CM, Boerwinkle E. Apolipoprotein E polymorphisms predict low density lipoprotein cholesterol levels

and carotid artery wall thickness but not incident coronary heart disease in 12,491 ARIC study participants. Am J Epidemiol. 2006; 164:342–348. [PubMed: 16760224]

## Highlights

- Discovery of variants in *APOE* gene region by pooled target capture sequencing
- Identification of longevity-associated variants in regulatory region of *APOE* gene
- Validation of longevity association of *APOE* ε2 allele in Ashkenazi population
- Association of longevity-associated allele with beneficial lipid profiles

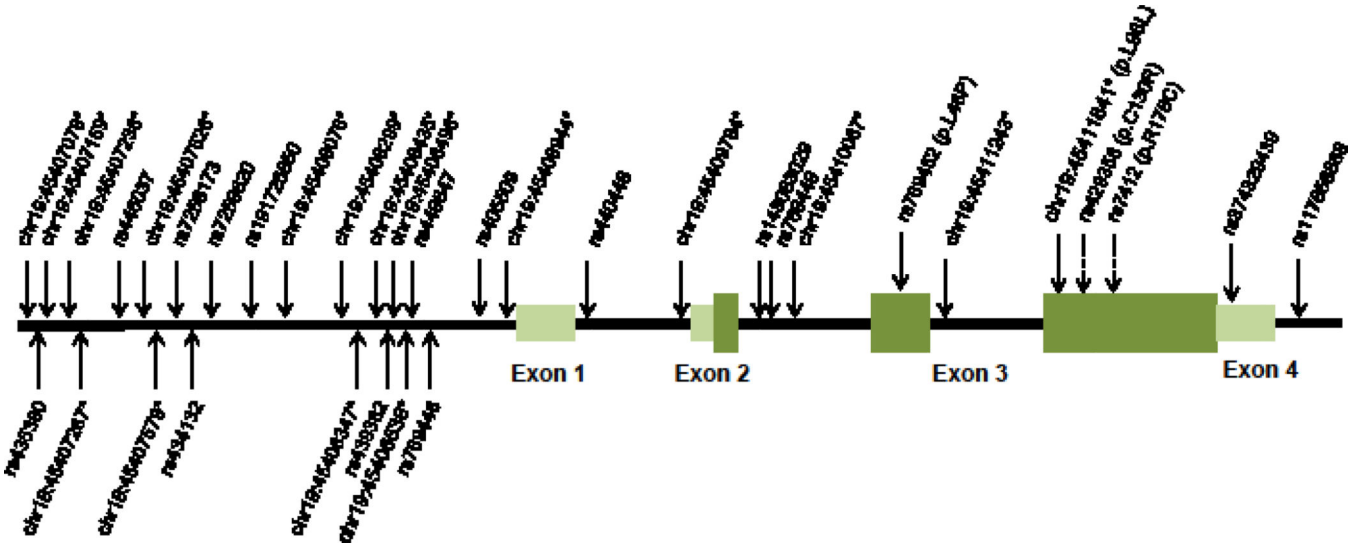


**Figure 1. Discovered variants in *APOE* gene from Pool-seq and genotyping in centenarians and controls**

The variants in *APOE* gene discovered from Pool-seq were indicated on gene structure. Discovered variants were named by NCBI rsSNP ID for known variants and chromosomal coordinates of human genome (hg19) for novel variants not reported on database. Novel variants were indicated by asterisk and variants for genotyping were indicated as dotted arrow.

**Table 1**

Association analysis of *APOE* genotypes and alleles with longevity and lipid profile.

| Type | Frequency (centenarians n=438, controls n=515) | | | | LDL level | | |
|---|---|---|---|---|---|---|---|
| | Centenarians (%) | Controls (%) | OR (95% CI) | p-value | Type | The rest | p-value |
| ε2 | 86 (9.82) | 62 (6.02) | 1.70 (1.21–2.39) | **0.003** * | 91.7+/−5.26 | 109+/−1.51 | **0.002** * |
| ε3 | 732 (83.6) | 875 (85) | 0.90 (0.70–1.15) | 0.412 | 108+/−1.57 | 102+/−3.67 | 0.11 |
| ε4 | 58 (6.62) | 93 (9.03) | 0.71 (0.51–1.00) | 0.061 | 112+/−5.09 | 107+/−1.52 | 0.41 |
| ε2/ε2 | 4 (0.91) | 2 (0.39) | 2.36 (0.43–13.0) | 0.422 | 74.5+/−21.6 | 108+/−2.07 | 0.13 |
| ε2/ε3 | 72 (16.4) | 52 (10.1) | 1.75 (1.20–2.57) | **0.005** * | 93.9+/−5.75 | 109+/−2.19 | **0.01** * |
| ε2/ε4 | 6 (1.37) | 6 (1.17) | 1.18 (0.38–3.68) | 0.780 | 94+/−27.8 | 108+/−2.08 | 0.63 |
| ε3/ε3 | 309 (70.5) | 371 (72) | 0.93 (0.70–1.23) | 0.616 | 109+/−2.4 | 103+/−3.86 | 0.15 |
| ε3/ε4 | 42 (9.59) | 81 (15.7) | 0.57 (0.38–0.85) | **0.005** * | 113+/−5.63 | 107+/−2.2 | 0.30 |
| ε4/ε4 | 5 (1.14) | 3 (0.58) | 1.97 (0.47–8.29) | 0.481 | 109+/−19.7 | 107+/−2.09 | 0.92 |

Bold numerals with asterisk indicate p-value < 0.05.