# Unique Lipoprotein Phenotype and Genotype Associated With Exceptional Longevity

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**Context** Individuals with exceptional longevity have a lower incidence and/or significant delay in the onset of age-related disease, and their family members may inherit biological factors that modulate aging processes and disease susceptibility.

**Objective** To identify specific biological and genetic factors that are associated with or reliably define a human longevity phenotype.

**Design, Setting, and Participants** In a case-control design, 213 Ashkenazi Jewish probands with exceptional longevity (mean [SD] age, 98.2 [5.3] years) and their offspring (n=216; mean [SD] age, 68.3 [6.7] years) were recruited from 1998 to 2002, while an age-matched control group of Ashkenazi Jews (n=258) and participants from the Framingham Offspring Study (n=589) were accepted as control groups.

**Main Outcome Measures** Detailed questionnaires, physical examination, and blood samples were taken, including assessment of lipids and lipoprotein subclass levels and particle sizes by proton nuclear magnetic resonance. Samples were also genotyped for the codon 405 isoleucine to valine (I405V) variation in the cholesteryl ester transfer protein (*CETP*) gene, which is involved in regulation of lipoprotein and its particle sizes.

**Results** High-density lipoprotein (HDL) and low-density lipoprotein (LDL) particle sizes were significantly higher in probands compared with both control groups ($P=.001$ for both), independent of plasma levels of HDL and LDL cholesterol and apolipoprotein A1 and B. This phenotype was also typical of the proband's offspring but not of the age-matched controls. The HDL and LDL particle sizes were significantly larger in offspring and controls without hypertension or cardiovascular disease, ($P=.001$ and $P=.008$, respectively). Furthermore, lipoprotein particle sizes, but not plasma LDL levels, were significantly higher in offspring and controls without the metabolic syndrome ($P<.001$). Probands and offspring had a 2.9- and 3.6-fold (in men) and 2.7- and 1.5-fold (in women) increased frequency, respectively, of homozygosity for the 405 valine allele of *CETP* (VV genotype), respectively, compared with controls ($P<.001$ for both). Those probands with the VV genotype had increased lipoprotein sizes and lower serum *CETP* concentrations.

**Conclusions** Individuals with exceptional longevity and their offspring have significantly larger HDL and LDL particle sizes. This phenotype is associated with a lower prevalence of hypertension, cardiovascular disease, the metabolic syndrome, and increased homozygosity for the I405V variant in *CETP*. These findings suggest that lipoprotein particle sizes are heritable and promote a healthy aging phenotype.

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INDIVIDUALS WITH EXCEPTIONAL LONgevity have been generally spared from major age-related diseases that are responsible for most deaths in elderly persons, such as cardiovascular disease (CVD), diabetes mellitus, Alzheimer disease, and cancer.[1] Various studies suggest that genetic determinants of exceptional longevity are highly heritable.[2,3] Siblings of centenarians have an 8- to 17-fold higher probability of living past the age of 100 years, accounting for only approximately 1 of 10000 individuals in the general population.[2,3] The offspring of long-lived parents have an approximately 50% lower prevalence of hypertension, diabetes mellitus, myocardial infarction, and stroke/transient ischemic attacks compared with age-matched control groups.[3] Furthermore, at least 1 study linked a locus on chromosome 4 to exceptional longevity.[4] Identification of biological markers and genes that are conducive to exceptional longevity may provide insights into mechanisms that protect from a host of common diseases and/or slow the biological processes of aging.

Longevity genes have been demonstrated in other species but the relevance to humans is controversial.[1] In contrast, rodent models of aging and ag-

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ing-related diseases may be more relevant to humans. Expression of human cholesteryl ester transfer protein (*CETP*) in rats, a species that commonly lacks this gene, leads to combined hyperlipidemia, coronary heart disease, and decreased survival, making *CETP* a strong candidate gene for human aging.[5] Cholesteryl ester transfer protein is involved in the regulation of reverse cholesterol transport and high-density lipoprotein (HDL) levels. Indeed high levels of low-density lipoprotein (LDL) cholesterol and low levels of HDL cholesterol are correlated with increased incidence of CVD in humans, although other factors may contribute to accelerated aging through effects on vascular wall, cancer, or other mechanisms.[1] Although lipoprotein levels are not consistently unusual in centenarians, this does not rule out its potential role in promoting longevity, because the levels may be different at the end of life in centenarians than they were at earlier ages.

We developed a novel study design in a genetically homogeneous founder population to identify the biological and genetic underpinnings of exceptional longevity by studying Ashkenazi Jewish families defined by long-lived probands. In addition, we recruited the offspring of probands and an age-matched control group, hypothesizing that the former may have inherited certain biological protective factors that could be more easily discerned at younger ages if compared with an age-matched control group.

## METHODS

### Study Design and Participants

In a case-control study, Ashkenazi Jews were recruited as described elsewhere.[3,6,7] This population derived from an undetermined small number (estimated to be in the several thousands) of founders. External factors, including ecclesiastical edicts prohibiting all social contact with Jews, the Crusades, the establishment of the Pale of Settlement, numerous Pogroms, and ethnic bigotry, resulted in the social isolation and inbreeding of the Ashkenazi Jews. This history resulted in both cultural and genetic homogeneity and has made this population useful for identification of several genes, the breast cancer gene being a prominent example.[8] Two hundred thirteen probands with exceptional longevity (157 women and 56 men; mean [SD] age, 98.2 [5.3] years; range, 95-107 years; 48% were >100 years) were recruited to participate in the study, which was conducted from 1998 to 2002. The participants' ages were defined by birth certificates or dates of birth as stated on passports. Probands were required to be living independently at 95-years-old as a reflection of good health, although at the time of recruitment they could be at any institution or level of dependency. In addition, probands had to have a first-degree offspring who was willing to participate in the study. The offspring group consisted of 122 women and 94 men (mean [SD] age, 68.3 [6.7] years; range, 51-89 years). We used 2 different control groups. The first control group consisted of spouses of the offspring (n=75; mean [SD] age, 70.2 [10.2] years; 53% women). Fewer spouses of offspring than offspring of probands were recruited because 22 spouses had died, 18 offspring were divorced or separated, 10 spouses were not Ashkenazi Jews, and 55 spouses elected not to participate. The first control group also consisted of 183 age-matched Ashkenazi Jewish controls recruited from the Einstein Aging Study[9] (mean [SD] age, 71.3 [9.1] years; 57% women) for a total of 258 participants. Of this first control group, 2 participants were excluded from the analysis because their parents had lived to be 98 and 102 years. A second control group consisted of 589 age-matched white participants enrolled in the Framingham Offspring Study (mean [SD] age, 67.8 [3.5] years; 48% women), a community-based cohort. Given the low prevalence of exceptional longevity in the general population, we reasoned that the Framingham controls are not likely to have a family history of exceptional longevity or to carry longevity genes or phenotypes.

Informed written consent was obtained in accordance with the policy of the committee on clinical investigations of the Albert Einstein College of Medicine, New York, NY.

### Clinical Evaluation

A research nurse visited the probands in the morning to draw a venous blood sample, obtain a medical history, measure height and weight, and perform a physical examination. Health histories were obtained using a standardized questionnaire. At that visit, the offspring and the participating spouses underwent similar evaluations, as previously described.[3,6,7] All blood samples were processed at the General Clinical Research Center at Albert Einstein College of Medicine.

We followed the National Cholesterol Education Program (Adult Treatment Panel III) guidelines,[10] defining the metabolic syndrome as the presence of 3 or more of the 5 risk factors: increased waist girth (>94 cm for women, 102 cm for men), increased blood pressure (>130/85 mm Hg or treatment for hypertension), increased fasting glucose (>110 mg/dL [>6.11 mmol/L] or drug treatment for diabetes), low plasma HDL cholesterol (<40 mg/dL [<1.04 mmol/L]), and elevated fasting triglycericde levels (>150 mg/dL [>1.70 mmol/L]).

### Lipids and Lipoproteins

Total plasma cholesterol, triglycerides, HDL, LDL, very LDL, and apolipoprotein A-I and B concentrations for Ashkenazi participants were performed by standard automated methods at the clinical laboratories of Montefiore Medical Center, Bronx, NY. The same lipid measurements were performed on fasting plasma samples from the Framingham Offspring Study as previously described.[11] The LDL and HDL subclass levels and mean particle sizes were determined for all participants by nuclear magnetic resonance (NMR) spectroscopy at LipoScience Inc (Raleigh, NC) as previously described.[12,13] Each NMR measurement produces the concentrations of 3 LDL

subclasses and 5 HDL subclasses of varying size. From the LDL and HDL subclass levels are calculated weighted-mean LDL and HDL particle sizes (nm diameter) and LDL particle concentrations (nmol/L). Lipoprotein subclasses were grouped as large LDL (21.3-23.0 nm), intermediate LDL (19.8-21.2 nm), small LDL (18.3-19.7 nm), large HDL (8.8-13.0 nm), intermediate HDL (8.2-8.8 nm), and small HDL (7.3-8.2 nm). Although the LDL sizes ranged from 18.3 to 23.0 nm and HDL sizes from 7.3 to 13.0 nm, small changes within this range are associated with marked clinical differences in CVD risks.

The LDL and HDL subclass distributions and particle sizes determined by NMR are highly correlated with those measured by gradient gel electrophoresis and density gradient ultracentrifugation.[14,15] The analytical reproducibility (given by the coefficient of variability) of LDL and HDL size is less than 0.5%,[12] and the stability on repeated drawing for LDL size was 0.9% and for HDL size was 1.1%.

### *CETP* Genotyping and Concentrations

Lipoprotein sizes are largely determined by hepatic lipase and *CETP*, in addition to other possible pathways, therefore providing the rationale to examine variation in these genes. We sequenced the promoter region of hepatic lipase gene in 100 centenarians compared with controls and did not find different frequencies in known and unknown polymorphic markers in this region. We then genotyped several known *CETP* polymorphic markers: –631 C/A (NCBI dbSNP rs1800776) and –629C/A (rs1800775) in the promoter; codon 405 isoleucine to valine (I405V) (rs5882) and D442G (rs2303790) in exons 14 and15, respectively; and G/A A multilocus polymerase chain reaction–based assay in the first nucleotide in intron 14 was used to genotype these polymorphisms.[16] Briefly, DNA was amplified using a multiplex reaction containing biotinylated primer pairs. Amplified fragments within each polymerase chain reaction product pool were then detected colorimetrically with sequence-specific oligonucleotide probes immobilized in a linear array on nylon membranes stripes. Probe specificities had previously been confirmed by sequencing and through use of DNA genotyped independently through other methods such as restriction length polymorphism analysis.[16] The CETP concentrations in human serum were measured by ELISA (Wako Chemicals USA Inc, Richmond, Va).

### Statistical Analyses

Pairwise crude comparisons of lipid levels and lipid particle sizes among the study groups were performed by using the Mann-Whitney *U* test because the distributions were skewed. Because of strong correlations among the lipid variables, a multivariate analysis was also needed to disentangle the relationships between the different lipid variables and study group membership. We conceptualized the study groups as forming an ordered set, with probands containing the highest prevalence, offspring an intermediate prevalence, and controls the lowest prevalence of longevity-promoting genes and phenotypes. Therefore, we used ordered logistic regression to determine which lipid variables exhibited the strongest ordinal association with study group membership. Study group membership (1 = control, 2 = offspring, 3 = proband) was the dependent variable, and LDL, HDL, LDL-particle size, and HDL-particle size were the independent variables. In interpreting the results of this analysis, direct comparison of the coefficients of the independent variables is not meaningful because they are measured in different units. The corresponding *z* statistics are all dimensionless and directly commensurable, and were used as measures of the ordinal association between the lipid variables and study group membership. Calculations were performed by using SAS version 6.12 (SAS Institute, Cary, NC) and Stata version 8.0 (Stata Corp, College Station, Tex). Narrow sense heritability ($h^2$) was estimated from the slope of the linear regression of the traits of each parent on the mean value of offspring.[17] For a comparison of the difference in *CETP*-I405V genotype frequency between the groups, Hardy-Weinberg equilibrium was tested and the $\chi^2$ test was performed. $P<.05$ was considered the threshold for statistical significance. Data are expressed as mean (SD or SE).

## RESULTS

### Lipoprotein Properties in Families With Exceptional Longevity

Because the extreme old age of the probands preempts a proper comparison (control) group, we also recruited the offspring of probands, some of whom presumably inherited longevity genes and thus should manifest the longevity phenotype. Candidate longevity phenotypes could then be compared between the offspring and age-matched Ashkenazi controls or non-Ashkenazi participants of the Framingham Offspring Study, neither of which are known to have longevity genes. There were no significant differences between the groups for routine blood chemistries, including electrolytes, liver function, and kidney function tests. Body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters) was similar between offspring and the Ashkenazi control groups ($P=.75$ and $P=.21$ in women and men, respectively), both of which were significantly higher than in probands (TABLE 1). Typical measures of lipoproteins, including total cholesterol, HDL, LDL, and triglycerides were quite similar in probands compared with control group as well as between offspring and control groups. Only total cholesterol and LDL levels were lower in female probands vs control groups, and HDL levels were significantly higher in female offspring compared with Ashkenazi control groups. In contrast, there were marked differences between groups in lipoprotein particle sizes as determined by NMR. The HDL and LDL particle sizes in probands were markedly higher compared with both control groups ($P=.001$

and $P=.001$ in women and men, respectively). As in their parents, offspring of exceptional longevity probands had significantly larger sizes of their LDL and HDL particles compared with their age-matched controls ($P<.001$ for both), although the difference in HDL particle size was not as high in men.

The plasma HDL frequency distribution was approximately normal in women (FIGURE 1) and men. However, offspring of probands had a skewed distribution in which 46% of the female offspring and 42% of male offspring had plasma HDL levels that were 1 SD above the mean. Similarly, we found a bimodal frequency distribution of HDL particle size with strikingly different distribution of LDL particle size in offspring of probands compared with control groups. These distributions suggest that only a subset of offspring carry the high HDL trait, which may have been inherited in these individuals. Additionally, this distribution could represent a survival effect. Trends for men were similar, although mean HDL and LDL particles size were lower than in women (data not shown).

Differences among the probands, offspring, and control groups in the proportions of total LDL and HDL contributed to by the large and small subclasses of these lipoproteins are shown in FIGURE 2. In probands, the large HDL and LDL subclasses accounted for a much higher proportion of total HDL and LDL than in the control groups, whereas the relative amounts of small HDL and LDL were much less than in control groups. Similar trends were observed in the offspring.

Examining the relationship between lipoprotein particle sizes and age in our sample, we found that both LDL and HDL particle sizes are larger for offspring of probands than in the control group across all ages examined (FIGURE 3). The same relationship was apparent when men and women were considered separately. Lipoprotein particle sizes were relatively constant until 80 to 85 years, after which particle sizes increased dramatically. This suggests more survival to older ages in in-

**Table 1.** Lipoprotein Properties in Families With Exceptional Longevity

| | Mean (SD) | | | | *P* Value | | | |
|---|---|---|---|---|---|---|---|---|
| | Proband | Offspring | Ashkenazi Control | Framingham* Control | Proband vs Ashkenazi Control | Proband vs Framingham Control | Offspring vs Ashkenazi Control | Offspring vs Framingham Control |
| **Women** | | | | | | | | |
| No. of participants | 157 | 122 | 147 | 276 | | | | |
| Body mass index† | 22.7 (3.3) | 24.9 (3.7) | 24.7 (3.3) | 26.7 (5.1) | .001 | .001 | .75 | .001 |
| Lipoprotein levels, mg/dL | | | | | | | | |
| Total cholesterol | 204 (40) | 227 (37) | 222 (37) | | .006 | | .22 | |
| LDL | 117 (35) | 128 (35) | 132 (35) | | .02 | | .27 | |
| ApoB | 96 (22) | 104 (24) | 100 (24.6) | | .35 | | .34 | |
| HDL | 56 (15) | 70 (17) | 59 (17) | | .17 | | .001 | |
| ApoA1 | 151 (29) | 186 (27) | 172 (31) | | .001 | | .001 | |
| VLDL, mg/dL | 73 (38) | 75 (47) | 79 (41) | 78 (42) | .21 | .22 | .50 | .51 |
| Lipoprotein particle size, nm | | | | | | | | |
| LDL | 21.5 (0.5) | 21.5 (0.5) | 21.0 (0.73) | 21.0 (0.5) | .001 | .001 | .001 | .001 |
| HDL | 9.55 (0.47) | 9.38 (0.46) | 9.18 (0.51) | 9.35 (0.43) | .001 | .001 | .001 | .56 |
| LDL particle concentration, nmol/L | 1079 (341) | 1190 (390) | 1135 (416) | 1576 (432) | .41 | .001 | .40 | .001 |
| **Men** | | | | | | | | |
| No. of participants | 56 | 94 | 111 | 309 | | | | |
| Body mass index† | 23.3 (2.6) | 26.7 (3.3) | 25.8 (3.1) | 26.9 (4.5) | .001 | .001 | .21 | .80 |
| Lipoprotein levels, mg/dL | | | | | | | | |
| Total cholesterol | 183 (36) | 196 (36) | 187 (33) | | .43 | | .03 | |
| LDL | 105 (29) | 112 (32) | 107 (27) | | .61 | | .20 | |
| ApoB | 91 (19) | 95 (21) | 88 (17) | | .41 | | .06 | |
| HDL | 50 (17) | 53 (15) | 47 (14) | | .28 | | <.001 | |
| ApoA1 | 131 (24) | 148 (29) | 143 (29) | | .04 | | .37 | |
| VLDL | 71 (37) | 77 (45) | 74 (31) | 93 (49) | .60 | .001 | .59 | .01 |
| Lipoprotein particle size, nm | | | | | | | | |
| LDL | 21.3 (0.6) | 21.1 (0.78) | 20.9 (0.59) | 20.7 (0.5) | .001 | .001 | .01 | .001 |
| HDL | 9.39 (0.57) | 9.09 (0.47) | 8.88 (0.35) | 9.04 (0.38) | .001 | .001 | .05 | .26 |
| LDL particle concentration, nmol/L | 1019 (246) | 1077 (336) | 949 (247) | 1589 (385) | .22 | .001 | .02 | .001 |

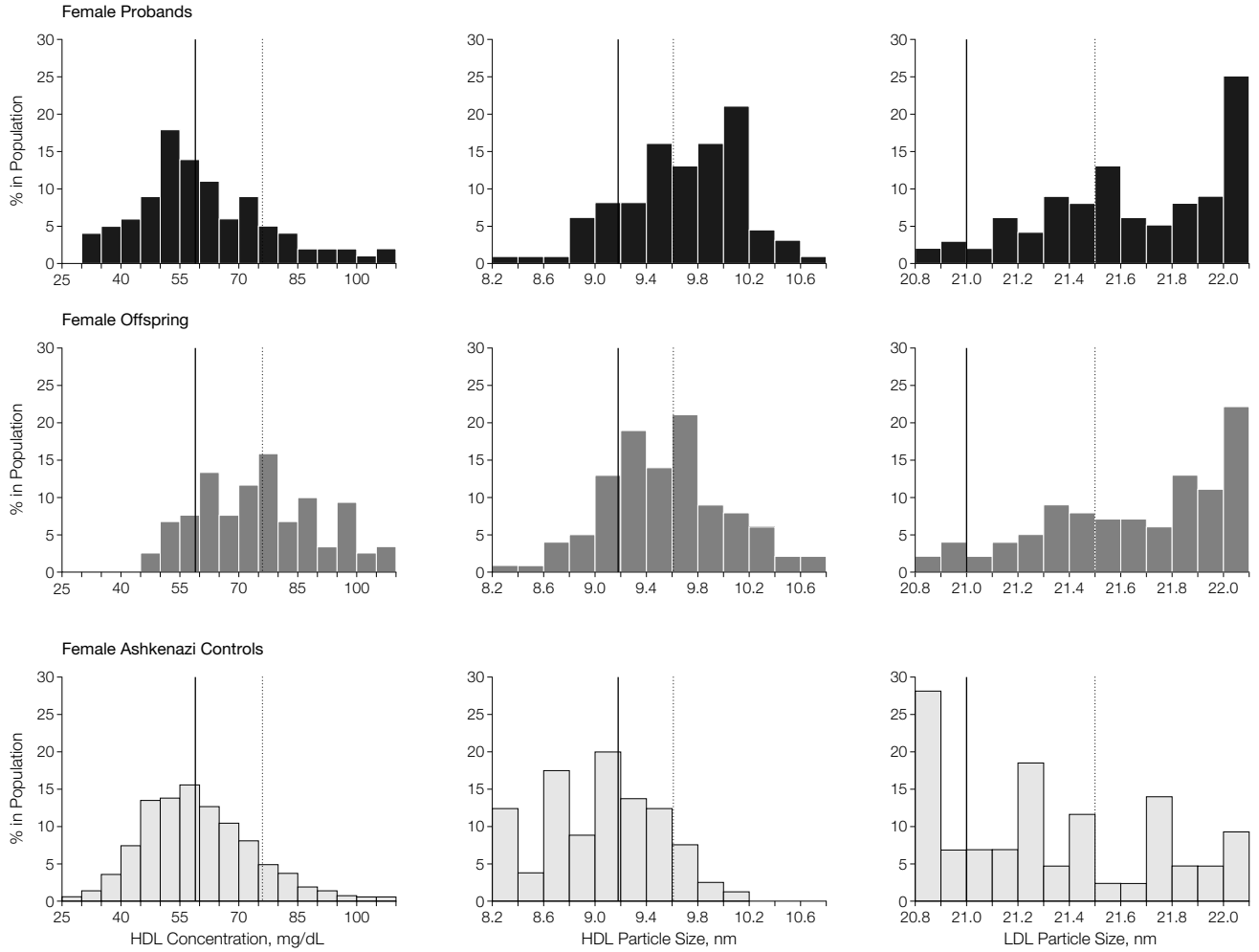
Abbreviations: Apo, apolipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.
SI conversion factors: To convert cholesterol, HDL, LDL, and VLDL levels to mmol/L, multiply by 0.0259.
*The measurements of the Framingham Offspring Study were not obtained at the same laboratory as the other groups and thus were not used for comparison.
†Calculated as weight in kilograms divided by the square of height in meters.

dividuals who have larger HDL and LDL particle sizes.

To further examine the differences in lipid value distributions among the study groups, we performed a multivariate analysis to examine the effect of each lipid variable while controlling for the effects of the others. Study group membership is an ordinal-level variable, with controls having the least, offspring an intermediate amount, and probands the highest frequency of genes and phenotypes that promote longevity. Therefore, we performed an ordered logistic regression with study group membership as the dependent variable, and the 4 lipid variables as independent variables. Direct comparison of the regression coefficients is not meaningful because the independent variables are measured in different units. The corresponding $z$ scores, however, are dimensionless and commensurable. The $z$ scores (associated $P$ values) for the 4 lipid values were –1.48 ($P=.14$) for LDL cholesterol, 4.52 ($P<.001$) for LDL size, –4.68 ($P<.001$) for HDL cholesterol, and 5.77 ($P<.001$) for HDL size. The $z$ statistics rank HDL size as the strongest and LDL size as the next strongest determinant of group membership. High-density lipoprotein cholesterol also provided strong ordinal discrimination among the groups, but in the opposite direction, and LDL cholesterol provided weak nonsignificant discrimination in the opposite direction as well.

Finally, because proband variance was equal between sexes, although the offspring variance was not equal between sexes, heritability of lipoprotein traits was performed in the 2 offspring sexes separately. The $h^2$ of HDL size is 0.32 (SD, 0.16) in female and 0.70 (SD, 0.22) in male offspring ($P=.01$ and $P=.004$,

**Figure 1.** Frequency Distribution of Lipoprotein Properties in Female Probands, Offspring, and Controls




The frequency distribution of plasma high-density lipoprotein (HDL) concentration levels and particle sizes, and low-density lipoprotein (LDL) particle sizes in female probands, their offspring, and an Ashkenazi control population. To convert HDL concentration to mmol/L, multiply by 0.0259. The solid lines represent the mean and the dotted lines represent 1 SD of control.

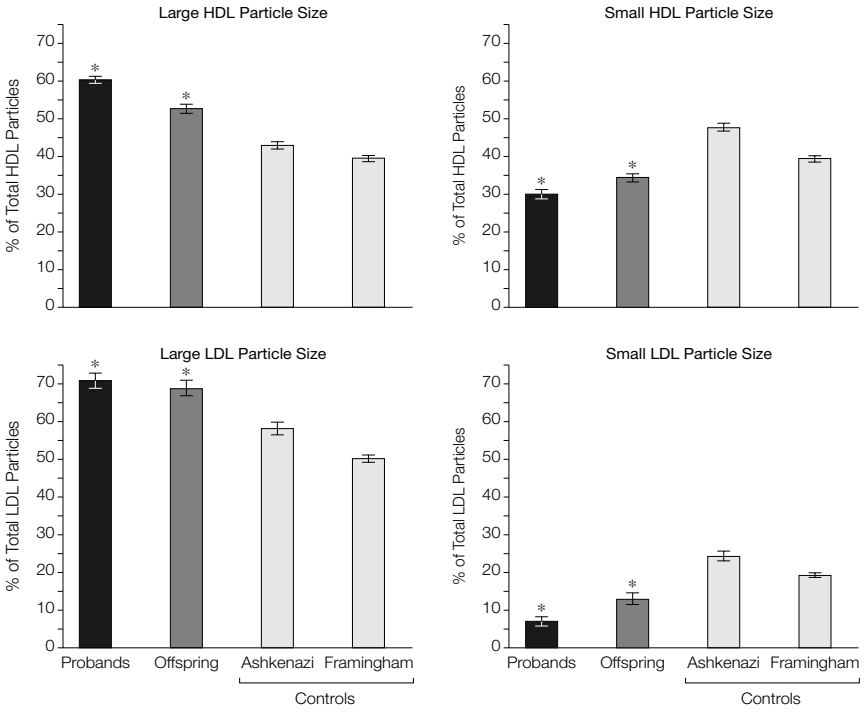
respectively). Similarly, $h^2$ of LDL size is 0.46 (SD, 0.20) in women and 0.6 (SD, 0.26) in men ($P=.003$ and $P=.006$, respectively). All were statistically significant, supporting a genetic linkage with lipoprotein sizes.

### LDL and HDL Particles Size in Relationship to Hypertension, CVD, and the Metabolic Syndrome

Because lipoprotein concentrations and size are important CVD risk factors, we next examined the relationship between lipoprotein particle size, hypertension, and the prevalence of CVD, defined as having a history of myocardial infarction, stroke, or transient ischemic attack in the combined group of our Ashkenazi offspring and control (TABLE 2). The Framingham Offspring Study and Einstein Aging Study were not included in this analysis because clinical evaluation for these CVD traits was performed differently. Significantly higher percentage of large HDL particles, HDL particle size, percentage of large LDL, and LDL particle size where observed in healthy participants compared with those with hypertension. Moreover, significantly higher percentage of large HDL particles, HDL particle size, percentage of large LDL, and LDL particle size were observed in healthy participants compared with those with CVD. Unexpectedly, LDL levels were lower in the hypertension and CVD groups, probably accounted for by use of cholesterol-lowering drugs (18% in the healthy group, 38% in the hypertension group, and 60% in the CVD group). Significantly lower levels of HDL in hypertension and CVD groups were observed compared with the healthy group, but neither very LDL nor triglyceride levels were significantly different between hypertension risk groups. In total, these findings suggest a possible link between the size of lipoprotein particles and age-related hypertension and CVD.

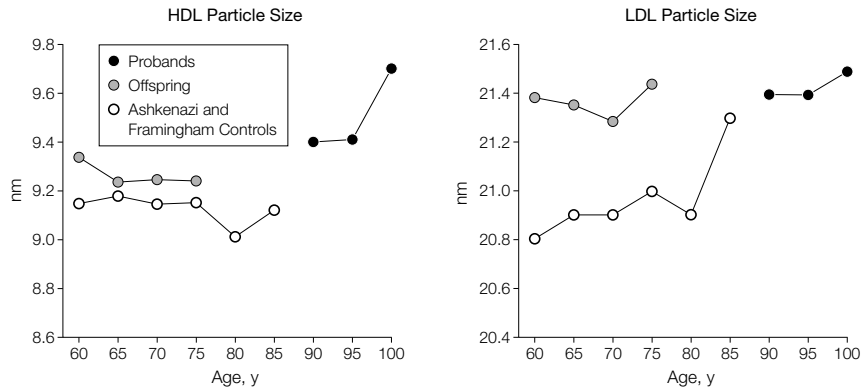
The metabolic syndrome (insulin resistance syndrome, syndrome X, dysmetabolic syndrome X)[10] is a risk factor for many causes of death.[18] We determined the frequency of metabolic syndrome according to the National Cholesterol Education Program III guidelines. Because the frequency of the metabolic syndrome increases with age, we would expect probands to have a much higher frequency of the metabolic syndrome than the younger control group. However, the frequency of the metabolic syndrome in probands was

**Figure 2.** Percentage of Large and Small HDL and LDL Particle Sizes in Long-Lived Probands, Offspring, and Ashkenazi and Framingham Controls




HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.
*$P<.001$ for probands vs Ashkenazi and Framingham controls and $P<.001$ for offspring vs Ashkenazi and Framingham controls for both large and small HDL and LDL particle sizes.

**Figure 3.** HDL and LDL Particle Size as a Function of Age




Cross-sectional data from probands with exceptional longevity aged 95 to 107 years (n=191), Ashkenazi and Framingham control groups combined aged 60 to 95 years (n=878), and their offspring aged 60 to 80 years (n=206). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

44%, similar to a frequency of 39% in the much younger control group. Offspring had a significantly lower frequency of the metabolic syndrome (26%, $P=.03$ vs control), although these groups were well matched for BMI and age. We further tested whether the participants without the metabolic syndrome have also larger lipoprotein sizes (Table 2). Indeed, larger HDL and LDL particle sizes were apparent when age-matched participants with and without the metabolic syndrome were compared. This effect was not noted for LDL levels, because of the widespread use of statin therapy. Because reduced HDL level is one of the criteria for having the metabolic syndrome and is associated with HDL particle size, we repeated the analysis with participants whose metabolic syndrome was redefined by 3 criteria other than plasma HDL levels. Lipoprotein particle sizes were still significantly larger in those without the metabolic syndrome. These findings suggest a lower frequency of metabolic syndrome–related traits in participants genetically predisposed to longevity.

### Polymorphism in *CETP* Gene and Phenotype of Exceptional Longevity

Cholesteryl ester transfer protein has been shown to modulate HDL and LDL levels and sizes.[19-22] To determine if genetic variation in *CETP* might influence lipoprotein particle size or longevity, we analyzed several common single nucleotide polymorphisms of *CETP*. All of the single nucleotide polymorphisms examined were in Hardy-Weinberg equilibrium in probands, their offspring, and the Ashkenazi control groups (Framingham Offspring Study samples were not genotyped). The allele frequencies of –629 C/A and D442G in Ashkenazi control groups were 0.57 and 0, respectively. Neither allele or genotype frequencies of the –629 C/A variant differed significantly among probands, offspring, or Ashkenazi control groups, nor was this variant associated with lipoprotein levels or particle sizes. By contrast, allele frequencies of the I405V allele were 0.46, 0.43, and 0.29 in probands, offspring, and Ashkenazi control groups, respectively. Strikingly, the frequency of homozygosity for the codon 405 valine allele of *CETP* (VV genotype) was 24.8% in female and male probands compared with only 8.6% in Ashkenazi control groups. These differences were statistically significant in both men and women ($P<.001$ for both) (FIGURE 4).

The offspring of probands had a VV genotype frequency of 20.7% in women and men combined, intermediate be-

**Table 2.** Lipoproteins and Their Particle Sizes in Relation to Age-Related Diseases

| Offspring and Spouse of Offspring* | Mean (SE) | | | *P* Value | |
|---|---|---|---|---|---|
| | Healthy | Hypertension | Cardiovascular Disease | Healthy vs Hypertension | Healthy vs Cardiovascular Disease |
| No. of participants | 209 | 64 | 20 | | |
| HDL | | | | | |
| Concentration, mg/dL | 64.5 (0.09) | 57.1 (0.3) | 50.8 (0.9) | .004 | .003 |
| Large particle size, % of total | 54.5 (0.07) | 46.8 (0.3) | 41.3 (1.1) | .002 | .001 |
| Particle size, nm | 9.32 (0.01) | 9.07 (0.06) | 8.96 (0.03) | .001 | .001 |
| LDL | | | | | |
| Concentration, mg/dL | 120.3 (2.6) | 116.9 (3.6) | 104.1 (3.9) | .20 | .03 |
| Large particle size, % of total | 66.5 (2.2) | 57.6 (2.5) | 43.3 (4.5) | .02 | .001 |
| Particle size, nm | 21.4 (0.03) | 21.1 (0.04) | 20.8 (0.05) | .008 | .001 |
| VLDL concentration, mg/dL | 80.2 (3.4) | 93.7 (5.3) | 114 (6.5) | .10 | .05 |

| Offspring and Spouse of Offspring† | Mean (SE) | | | *P* Value | |
|---|---|---|---|---|---|
| | Healthy | The Metabolic Syndrome | The Metabolic Syndrome (Excluding HDL Criteria) | Healthy vs The Metabolic Syndrome | Healthy vs The Metabolic Syndrome (Excluding HDL Criteria) |
| No. of participants | 221 | 47 | 25 | | |
| HDL | | | | | |
| Concentration, mg/dL | 63.2 (1.14) | 46.6 (1.75) | 46.8 (4.98) | <.001 | .002 |
| Large particle size, % of total | 66.9 (1.58) | 42 (3.94) | 40.89 (2.9) | <.001 | <.001 |
| Particle size, nm | 9.28 (0.03) | 8.88 (0.05) | 8.95 (0.07) | <.001 | .001 |
| LDL | | | | | |
| Concentration, mg/dL | 122 (2.14) | 112.9 (5.4) | 114.9 (6.43) | .09 | .32 |
| Large particle size, % of total | 56.5 (0.9) | 39.7 (2.06) | 51.3 (2.23) | <.001 | <.001 |
| Particle size, nm | 21.33 (0.04) | 20.76 (0.11) | 20.97 (0.13) | <.001 | .01 |
| VLDL concentration, mg/dL | 66.8 (2.5) | 106.4 (6.3) | 108 (7.2) | <.001 | <.001 |

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.
SI conversion factor: To convert HDL, LDL, and VLDL concentrations to mmol/L, multiply by 0.0259.
*Healthy, hypertension (>130/85 mm Hg or treatment for hypertension), or history of cardiovascular disease (history of either myocardial infarction or stroke/transient ischemic attack).
†Healthy, the metabolic syndrome (by National Cholesterol Education Program [Adult Treatment Panel III] criteria[11]), and the metabolic syndrome by 3 criteria excluding HDL.

tween probands and control groups and also significantly higher than in control groups ($P=.004$).

In principle, one cannot calculate the attributable risk of VV genotype for longevity from a case-control study because it is derived from incidence rates. However, a reasonable approximation can be calculated by using the estimation formula:

$$\text{Population Attributable Risk} = P \times OR(1 + P \times OR),$$

where P is the prevalence of VV homozygosity in the population and OR is the odds ratio associating VV homozygosity with longevity. This estimation formula is valid so long as the outcome (longevity) is rare in the population (approximately 1 of 5000-10000). In our data, the OR (combining men and women) is 3.56. We do not have a direct observation of population prevalence of VV homozygosity in our data. However, it is reasonable to take the control groups as a proxy for the population as a whole because the number of people who will ultimately survive to longevity is a very small proportion of the population. Our estimate of the prevalence of VV homozygosity is 0.09, and applying the formula, population attributable risk fraction is 18.1.

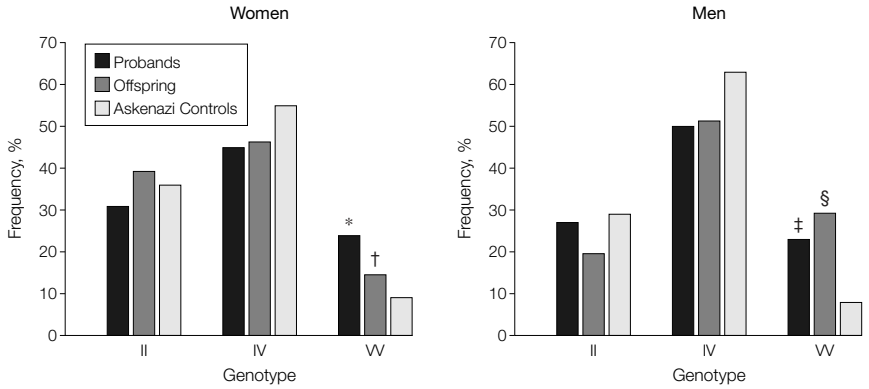
We also assessed the relationship between *CETP* I405V genotype and lipoprotein particle sizes and CETP activity in the control, offspring, and proband groups, respectively. The VV phenotype was associated with significantly larger LDL and HDL particle sizes (TABLE 3). Furthermore, participants with the VV genotype had 17% lower *CETP* concentrations compared with those with II or IV genotype, and HDL level and CETP levels were negatively correlated ($r=-0.29$; $P=.03$; Spearman ρ). These findings suggest a survival advantage for individuals with the VV genotype, perhaps mediated through decreased levels of *CETP* and its effects on lipoproteins and their particle sizes.

## COMMENT

Participants with exceptional longevity escape or delay many age-related diseases, including CVD, dementia, infections, and cancer. The longevity phenotype is likely to be one that involves diverse biological processes and protects from a number of age-related diseases, and these processes may be different than suggested from studies of other species.[23] This study demonstrates to our knowledge the first time that families with exceptional longevity have markedly larger particle sizes of HDL and LDL, which are largely independent of the absolute levels of lipoproteins and apolipoproteins. This particular phenotype is associated with a lower prevalence of hypertension and CVD and the metabolic syndrome in their offspring compared with appropriately age-matched control groups, supporting a functional role for lipoproteins in promoting survival to very old age. The pattern of distribution of lipoprotein sizes, their marked heritability in the offspring, and the markedly increased frequency of homozygosity for the codon 405 valine *CETP* allele support a genetic component to this phenotype and exceptional longevity.

Does lipoprotein particle size play a direct biological role in successful aging and the longevity phenotype, or are these measures simply markers of the longevity phenotype? Although our

**Figure 4.** Genotype Frequencies of I405V *CETP* in Long-Lived Probands, Their Offspring, and Ashkenazi Controls




The frequency of homozygosity for the codon 405 isoleucine to valine (I405V) allele of cholesteryl ester transfer protein (*CETP*) in female and male probands (n=156), offspring (n=163), and Ashkenazi controls (n=129). The frequency of the VV genotype is 2.4- and 2.9-fold increased in offspring and probands, respectively, compared with Ashkenazi controls. *$P=.001$; †$P=.20$; ‡$P=.004$; §$P<.001$, indicating differences between probands and offspring vs Ashkenazi controls for women and men.

**Table 3.** *CETP* I405V Genotype and Lipoprotein Characteristic and Plasma *CETP* Levels in Families With Exceptional Longevity and Control Groups

| Variable | *CETP* I405V Genotype, Mean (SD) | | | *P* Value (VV vs II Genotypes) |
|---|---|---|---|---|
| | VV | IV | II | |
| HDL | | | | |
| Concentration, mg/dL | 57 (18) | 55 (16) | 55 (16) | .53 |
| Large particle size, % of total | 56 (16) | 60 (14) | 60 (15) | .10 |
| Particle size, nm | 9.28 (0.56) | 9.09 (0.52) | 9.07 (0.48) | .02 |
| LDL | | | | |
| Concentration, mg/dL | 114 (35) | 120 (30) | 123 (34) | .16 |
| Large particle size, % of total | 67 (25) | 58 (24) | 56 (28) | .02 |
| Particle size, nm | 21.29 (0.67) | 20.98 (0.63) | 20.88 (0.81) | .002 |
| *CETP* concentration, µg/mL | 1.65 (0.59) | 1.92 (0.65) | 1.99 (0.72) | <.001 |

Abbreviations: *CETP*, cholesteryl ester transfer protein; HDL, high-density lipoprotein; I405V, codon 405 isoleucine to valine; LDL, low-density lipoprotein.
SI conversion factor: To convert HDL and LDL concentrations to mmol/L, multiply by 0.0259.

study cannot directly answer this question, we suggest that lipoprotein concentrations and particle sizes are excellent causal biological candidates. As is the case for HDL levels, HDL and LDL particle sizes are significantly larger in women than in men, and may explain in part why women have lower CVD incidence rates and have higher life expectancies than men. Small LDL particles penetrate more readily into arterial tissue, bind more tightly to arterial proteoglycans, are oxidized more rapidly than larger LDL particles, and are associated with endothelial dysfunction, all mechanisms involved in the development of CVD.[15,24] Therefore, large LDL particle size may be important in protecting the vascular bed from age-related atherosclerosis and thus promoting exceptional longevity. Similarly, small HDL particle size has been demonstrated in patients with CVD,[25] and some lipid-lowering drugs may protect from CVD by shifting the HDL particles to larger sizes that are similar to those observed in patients without CVD.[26] Increased HDL concentration and particle size are likely to underlie the beneficial effects of exercise on the cardiovascular system and other age-related phenotypes.[27] However, causality between HDL particle size and CVD has been debated because of its association with small LDL particles and increased triglycerides.

Until recently, HDL cholesterol was thought to exert its effects through reverse cholesterol transport. The ability of HDL to clear cholesterol from the endothelium and peripheral tissues may have systemic protective effects from lipotoxicity similar to that obtained in caloric-restricted rodents, whose life span is dramatically prolonged.[28] In addition to its role in lipid metabolism, other beneficial biological properties of HDL have been described, including anti-inflammatory, antioxidant, antiaggregatory, anticoagulant, and profibrinolytic activities, which are exerted by HDL particles and other components, through their interactions with several apolipoproteins, enzymes, and even specific phospholipids.[29,30] This complexity emphasizes that changes in the functionality of HDL, some through changes in mass or size, may have pleiotropic antiaging effects.

In population studies, there is a strong inverse correlation of plasma levels of LDL and very LDL to HDL levels and HDL and LDL particle size.[12,31] Our study shows that the levels of HDL and LDL, and their respective apolipoproteins and particle sizes, are correlated with each other. We used ordered logistic regression analysis to untangle these measures. Our analysis indicates that LDL and HDL particle sizes remained significant predictors of having longevity even after taking into account absolute levels of lipids, lipoproteins, and BMI. This large lipoprotein particle size phenotype is also evident in the offspring of long-lived probands. We interpret these data to indicate that lipoprotein particle size is an independent heritable predictor of longevity. Indeed, there is recent compelling evidence implicating LDL lipoprotein particle size as a stronger predictor of CVD than LDL levels.[31] Yet it is possible that the lipoprotein levels and not the particle sizes directly may be relevant for their protective actions, even if they do decline with aging.

In support of causal relationship between lipoprotein sizes and age-related diseases, offspring have significantly less hypertension and CVD, and lipoprotein sizes distinguish between those with and without these conditions. Insulin resistance and diabetes are associated with significantly lower HDL and LDL particle sizes.[32] The frequency of insulin resistance, which is the hallmark of the metabolic syndrome of aging, is significantly lower in the offspring. Furthermore, the incidence of insulin resistance in probands is much less than would be expected (approximately 50% after age 70 years).[18] The lipoprotein sizes reported in probands are significantly larger than these reported in a control group for the insulin resistance and diabetes study,[32] further supporting the clinical significance of relative small changes in their sizes and outcomes.

Because it has been shown that lipoprotein sizes are genetically determined,[33] we were interested in finding potential pathways involved in this phenotype. The biology of rare forms of *CETP* deficiency may be relevant to our observation of a marked increase in the frequency of homozygosity for the common codon 405 valine *CETP* allele in our families with longevity. Although homozygosity for this variant was observed in 24.8% of our probands, it was present at a frequency of only 8.6% in Ashkenazi Jewish control participants. Offspring of long-lived individuals also had a much higher frequency of the VV genotype compared with control groups. With analyses of 30 different single nucleotide polymorphisms, it could be argued that our positive finding for I405V *CETP* is the result of multiple comparisons. However, the level of statistical significance of this association ($P<.001$) maintains statistical significance even after rigorous correction for multiple comparisons. Furthermore, investigation of this variant was hypothesis driven based on known pathways of lipoprotein metabolism. Correction for multiple comparisons would be considered by many as overly conservative. To our knowledge, there is no other example of a polymorphic allele whose frequency was demonstrated to be so dramatically increased in centenarians and for which there is plausible biological mechanism to explain the association. Although the effect of the valine 405 allele on the structure and function of the CETP protein has not been studied, homozygosity for the valine allele has been shown to result in significantly decreased CETP concentrations,[34-36] as we demonstrated; and significantly decreased CETP activity,[37-39] as confirmed by Boekholdt and Thompson.[40] Furthermore, in 2 populations, each with more than 1000 participants, valine 405 homozygosity was associated with a 6.5-mg/dL or approximately 13% increase[41] and approximately 4% increase[42] in HDL cholesterol.

Although this VV homozygosity appears in less than 20% of the offspring, more than 40% of offspring have very

high levels and increased size of HDL. This discrepancy may be explained by other variants in *CETP* or in other genes that modulate lipoprotein particle concentrations and size. Other single nucleotide polymorphisms in the *CETP* genes did not differ in frequency between our groups. Moreover, we analyzed regulatory domains of hepatic lipase, which also regulates lipoprotein size, and failed to note any increased frequency of any of the variants we found. Recently, Arai et al[43] investigated more than 200 Japanese centenarians for association between a *CETP Taq*I polymorphism (but not the I405V polymorphism) as well as hepatic lipase polymorphisms, and did not demonstrate an increase in frequency in this population. Because *CETP* deficiency has not been previously associated with increased LDL size, additional genes that modulate LDL size or environmental factors are likely to be involved in the regulation of LDL particle sizes and may be necessary to enhance the probability of exceptional longevity. Whether lipoprotein particle size and the I405V *CETP* polymorphism are important predictors of longevity in other populations remains to be determined.

Case-control studies are subject to problems that sometimes limit their validity. However, in the case of genetic studies, these limitations are frequently overcome. A major problem affecting case-control studies is biased-recall ascertainment of exposure status. When the exposure is a genotype, however, this problem cannot arise. Similarly, the use of prevalent cases and control groups entails length-biased sampling; but with longevity as the outcome, once again the problem is nullified. Case-control studies cannot by themselves establish a causal relationship but with a genotype exposure, at least the possibility of reverse causality is entirely excluded and the exposure is unequivocally known to precede the outcome. The recruitment of cases and control groups from separate population groups is another common source of bias in case-control studies. In our situation, the controls were spouses or neighbors of the offspring, neither of which were enriched with longevity genes, and the cases were the offspring group who were children of the probands. Therefore, differences in environment, social, and economic influences are unlikely to arise. The additional use of the Framingham Offspring Study as a second control group with similar findings further supports the validity of our findings. A final limitation of the case-control design, which does affect this study, is that outcome incidence rates and relative risks cannot be estimated. Although we are aware of the limitations of a case-control study, this approach in families with exceptional longevity and where genetic markers are tested may be the best possible approach.

n recent years, certain genetic manipulations in lower species have been shown to extend life-span by mechanisms that seem unlikely to be relevant in humans.[1] Our endeavor to study exceptional longevity in Ashkenazi Jews was based on evidence for strong inheritance of this phenotype.[1-4] However, the lack of good biological markers, which might provide mechanistic insights into exceptional longevity, has hampered systematic genetic analysis. The important findings of this work suggest pleiotropic vascular effects of lipoproteins with large particle sizes that are health promoting. Striking association of exceptional longevity with homozygosity for the valine 405 allele of *CETP* may explain in part the link between lipoprotein particle size and exceptional longevity. Further elucidation of the genetic and biological mechanisms that determine lipoprotein particle sizes may provide key insights into preventive and therapeutic interventions for several age-related diseases that impart significant morbidity and mortality to elderly individuals.

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