Original article

LDL cholesterol still a problem in old age? A Mendelian randomization study

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Abstract

Background: Observational studies in older subjects have shown no or inverse associations between cholesterol levels and mortality. However, in old age plasma low-density lipoprotein cholesterol (LDL-C) may not reflect the lifetime level due to reverse causality, and hence the risk may be underestimated. In the current study, we used an LDL genetic risk score (GRS) to overcome this problem.

Methods: A weighted GRS was created using 51 single nucleotide polymorphisms associated with LDL-C levels. The LDL GRS was calculated in three Dutch cohorts: the Leiden Longevity Study (LLS) ($n = 3270$), the Leiden 85-plus study ($n = 316$) and the Rotterdam Study ($n = 4035$). We assessed the association between the LDL GRS and LDL-C levels, chronological age, familial longevity and mortality.

Results: Up to 90 years of age, in each age stratum individuals with high LDL GRS had higher LDL-C levels ($P = 0.010$ to $P = 1.1 \times 10^{-16}$). The frequency of LDL-increasing alleles decreased with increasing age [$\beta = -0.021$ (SE = 0.01) per year, $P = 0.018$]. Moreover, individuals with a genetic predisposition for longevity had significantly lower LDL GRS compared with age-matched individuals of the general population [LLS nonagenarians vs > 90 years: $\beta = 0.73$ (SE = 0.33), $P = 0.029$, LLS offspring vs partners: $\beta = 0.66$ (SE = 0.23), $P = 0.005$]. In longitudinal analysis, high GRS was associated with increased

all-cause mortality in individuals > 90 years, with a 13% increased risk in individuals with the highest LDL GRS (*P*-trend $= 0.043$).

Conclusion: Results of the current study indicate that a genetic predisposition to high LDL-C levels contributes to mortality throughout life, including in the oldest old, and a beneficial LDL genetic risk profile is associated with familial longevity.

Key words: LDL-cholesterol, old age, genetic risk score, Mendelian randomization

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Key Messages

- Previous studies including older individuals suggest that the causal relation between LDL-C and (cardiovascular) disease is absent at old age.
- To overcome the potential influences of reverse causality and confounding, we used in the current study a LDL GRS as an instrumental variable.
- Our results indicate that a genetic predisposition to high LDL-C contributes to mortality throughout life, including in the oldest old.
- In addition, a beneficial LDL genetic risk profile is associated with familial longevity.

Introduction

Observational studies including middle-aged individuals have shown a positive association between cardiovascular disease and cholesterol levels.[1,2] In addition, lowering cholesterol levels with statins reduces the risk of cardiovascular disease at all ages.[1] However, at older ages above 75 years, the contribution of high cholesterol as a cardiovascular risk factor is controversial. Mortality from disease in old age has been shown to be independent of total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels,[3,4] whereas low total cholesterol levels have been associated with higher all-cause mortality in the oldest old.[3,5,6] At old age, LDL-C levels in plasma may not reflect lifetime LDL-C level, due to comorbidities.[3] This inverse health relation in old age raises the question whether lipid levels represent causal factors affecting cardiovascular/metabolic health at all ages. Fortunately, the use of genetic variants as an instrumental variable provides a possibility to investigate the associations free of biases such as reverse causality. In recent years, genome-wide association studies (GWAS) have identified several new genetic loci that are associated with lipoprotein levels.[7,8] The currently largest GWAS meta-analysis, including more than 188 000 individuals, found 157 loci to be associated with cholesterol levels.[8]

Characteristics of lipid metabolism have further been linked to human lifespan regulation by association with familial longevity. For example, offspring of long-lived individuals have larger LDL particle sizes compared with their spouses or age- and lifestyle-matched controls.[9,10,11]

Moreover, older people who carry the apolipoprotein E gene ε3/ε3 variant and have lower plasma levels of apoE, have a decreased mortality risk compared with carriers of the ε3/ε3 variant with high levels of ApoE.[12]

In this study we created a genetic risk score (GRS) based on single nucleotide polymorphisms (SNPs) associated with LDL-C levels.[8] Using this GRS as an instrumental variable, we evaluated the association between LDL-C and mortality in participants of three Dutch studies. In addition, we assessed the association between the LDL GRS and familial longevity.

Methods

Study populations

To assess the associations between the LDL GRS and the various outcomes, we made use of three Dutch cohort studies including 7634 participants: the Leiden Longevity Study (LLS), the Leiden 85-plus study and the Rotterdam Study (Figure 1). All cohorts had GWA data available and are briefly described here.

Leiden Longevity Study

For the LLS, long-lived siblings of European descent were recruited together with their offspring and the spouses of the offspring (partners). Families were included if at least two long-lived siblings were alive and fulfilled the age criterion of 89 years or older for men and 91 years or older for women, representing less than 0.5% of the Dutch
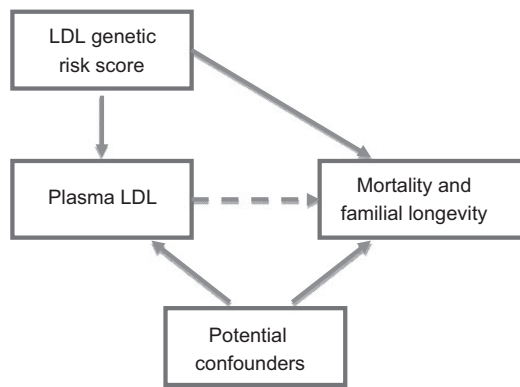
Figure 1. Graphical presentation of the research question in the current study.

population in 2001.[13] In total, 931 long-lived siblings with a mean age of 94 years (range 89–104), 1671 offspring (mean age 61 years, range 39–81) and 744 partners (mean age 60 years, range 36–79) were included. DNA from the participants of the LLS was extracted from samples at baseline using conventional methods.[14]

Leiden 85-plus study
Participants of the Leiden 85-plus study were inhabitants of Leiden, The Netherlands, who reached the age of 85 years between 1 September 1997 and 1 September 1999. There were no selection criteria on health, functioning or demographic characteristics. A total of 705 inhabitants reached the age of 85 years and a total of 599 individuals participated.[15] Individuals were visited at their place of residence and annual follow-up visits were performed until death or age 90 years. Information about mortality was available until 31 December 2009. The date of death was obtained from the civic registry of Leiden.

Rotterdam Study
The Rotterdam Study is a population-based cohort study including 7983 participants living in Ommoord, a district of Rotterdam, The Netherlands. All inhabitants aged 55 and over were invited to participate in the study ($n = 10\,275$). The Rotterdam Study started in the early 1990s, and periodical examinations were performed every 3–5 years. Analyses of this study are based on data from the third round of the study which was performed between 1997 and 1999 ($n = 4035$). The study was approved by the medical ethical committee of the Erasmus Medical Center and written informed consent was obtained from all participants.[16]

Lipoprotein levels

In offspring and partners from the LLS, non-fasting venous blood samples were taken. Total cholesterol, triglyceride and HDL cholesterol (HDL-C) levels were determined using fully automated equipment (the Hitachi Modular or the Cobas Inergra 800, both from Roche, Almere, The Netherlands).

In the Leiden 85-plus study, lipoprotein levels were obtained at the follow-up visit at age 90 years. Total cholesterol, triglyceride and HDL-C levels were analysed with fully automated computerized analysers (Hitachi 747 and 911, Hitachi, Tokyo, Japan).

In the Rotterdam Study, total cholesterol, HDL-C and triglyceride concentrations were measured from serum or plasma extracted from whole blood, using an automated enzymatic procedure (Boehringer Mannheim System).

In all three cohorts, LDL-C levels were calculated using the Friedewald equation.[17]

Genotyping

In the Leiden Longevity Study, genotyping was performed with Illumina Human660W-Quad and OmniExpress BeadChips (Illumina, San Diego, CA, USA). Individuals were removed if they showed a mismatch in gender or familial relatedness based on genotype and phenotype, leaving 928 nonagenarians, 1602 offspring and 740 partners for the analysis. In addition, SNPs which were not measured on both platforms and with a call rate < 0.95, MAF < 0.01 and $P_{HWE} < 10^{-4}$ were excluded, leaving 288 635 (nonagenarians) and 298 538 (offspring and partners) SNPs as input for the imputation. Imputation was performed separately for the LLS nonagenarians and LLS offspring and partners, using IMPUTE2 with reference HapMap Phase I + II CEU release 22 (hg18/build36).

In the Leiden 85-plus study, genotyping was performed with Illumina OmniExpress BeadChips (Illumina) in participants aged 90 years. Individuals were removed if they showed a mismatch in gender based on genotype and phenotype, leaving 316 individuals for the analysis. In addition, SNPs with a call rate < 0.95, MAF < 0.01 and $P_{HWE} < 10^{-4}$ were excluded, leaving 603 301 SNPs as input for the imputation. Imputation was performed using IMPUTE2 with reference HapMap Phase I + II CEU release 22 (hg18/build36).

In the Rotterdam Study, genotyping was conducted using the Illumina Infinium II HumanHap 550 K array among self-reported Caucasian individuals. Individuals were excluded if they had excess autosomal heterozygosity, mismatch between called and phenotypic gender or were recognized as being outlier with IBS clustering analysis. In addition, SNPs with an MAF $\leq 1\%$, $P_{HWE} < 10^{-5}$ or call rate $\leq 90\%$ were excluded, leaving 530 683 SNPs. Imputation was performed using the maximum likelihood method implemented in MaCH (version 1.0.15) with

reference to HapMap Phase I + II CEU release 22 (hg18/build36).

Weighted genetic risk score

To create the LDL GRS, we used the SNPs identified in the GWAS meta-analysis reported by the Global Lipids Genetics Consortium.[8] We included all 51 SNPs associated with LDL-C levels (and possibly with total cholesterol, HDL-C and/or triglycerides). Of all SNPs associated with LDL-C in the Global Lipids Genetics Consortium analysis, three SNPs (rs9411489, rs1801689 and rs6831256) were excluded since they were not available. To build the LDL GRS, we first determined the number (or dosage in the case of imputed SNPs) of unfavourable alleles for each individual, whereby the unfavourable allele was associated with higher LDL-C levels in the GWAS meta-analysis.[8] The number of unfavourable alleles was multiplied by the absolute effect size as published in the original paper.[8] Next, we calculated the GRS for each individual by summing the estimates (number of unfavourable alleles x absolute effect size) of all SNPs and divided it by the average of all effect sizes. In the final step, the GRS was rescaled into a percentage of the maximum number of risk alleles (individuals GRS / maximum GRS score) x 100%. For presentation purposes, the GRS percentage was divided into three approximately equal groups, using 48% and 52% as cutoff values. All *P*-values were assessed using the continuous level of the GRS percentage.

Statistical analysis

First, to assess the association between LDL GRS categories and LDL-C levels, we combined the data of general population subjects (LLS partners, Leiden 85-plus study and Rotterdam Study) and divided the individuals into age strata of 10 years. Data of LLS nonagenarians and offspring were excluded from this analysis since they have a genetic predisposition for longevity and to exclude possible familial effects. A general linear model was used adjusted for age, sex and cohort. Additional analyses were performed to adjust for HDL-C and triglyceride levels. The explained variance in LDL-C levels by the LDL GRS was assessed by calculating the R^2 per cohort using a linear regression model.

Second, we assessed the cross-sectional association between the LDL GRS and chronological age. Individual-level data from the LLS partners, Leiden 85-plus study and the Rotterdam Study were combined to have a wide variation in age range. A general linear model was used adjusted for sex and cohort. Additional analyses were performed using only the individuals aged ≥50 years

and ≥70 years, and to adjust for HDL-C and triglyceride levels.

Differences in LDL GRS between LLS nonagenarians and individuals ≥90 years, and LLS partners and offspring, were tested using a general linear model adjusted for age, sex and, if necessary, familial relations.

Finally, the longitudinal association between LDL GRS categories and mortality in individuals ≥90 years was assessed using Poisson analysis to calculate incidence rate ratios in each LDL GRS category. For this analysis, data of the LLS nonagenarians, Leiden 85-plus study and Rotterdam Study participants aged ≥90 years were used. Incidence rate ratios were adjusted for age, sex and, if necessary, cohort and familial relations. *P*-values were assessed using the continuous value of the LDL GRS.

All statistical analyses were performed using IBM SPSS Statistics program for Windows (Version 20.0, USA) and Stata/SE version 12.1 for Windows.

Results

The LDL GRS was calculated for 3270 participants (928 nonagenarians, 1602 offspring and 740 partners) of the Leiden Longevity Study, 316 participants of the Leiden 85-plus study and 4035 participants of the Rotterdam Study. In Table 1, the baseline characteristics are shown for the six age strata including the general population participants (LLS partners, Leiden 85-plus study and Rotterdam Study), the LLS nonagenarians and offspring. Baseline characteristics per cohort are provided in Supplementary Table 1 (available as Supplementary data at *IJE* online).

Up to 90 years of age, in each age stratum there was a linear association between the LDL GRS and LDL-C levels (Figure 2), with individuals in the highest LDL GRS group having the highest LDL-C level ($P = 0.010$ to $P = 1.1 \times 10^{-16}$). Associations between the LDL GRS and LDL-C levels in the separate cohorts are provided in Supplementary Figure 1 (available as Supplementary data at *IJE* online), showing a linear association in each cohort, except the Leiden 85-plus study. Additional adjustment for HDL-C and triglyceride levels did not change the observed associations (Supplementary Figures 2 and 3, available as Supplementary data at *IJE* online). The LDL GRS explained 6.8% of the variance in LDL-C levels in the LLS offspring, 5.1% in the LLS partners, 0.5% in the Leiden 85-plus study and 4.5% in the total Rotterdam Study.

The next step was to assess the association between the LDL GRS and chronological age. Figure 3 shows the cross-sectional relation between the LDL GRS and age. With increasing age, the LDL GRS decreased. Regression analysis showed a significant association between LDL GRS

Table 1. Baseline characteristics

Characteristics	Age strata (years)						Leiden Longevity study	
	<50	50-60	60-70	70-80	80-90	>90	Offspring	Nonagenarians
N	87	327	1859	1739	655	424	1602	928
Age (years)	45.8 (4.3)	55.7 (2.6)	65.7 (2.5)	74.7 (2.9)	83.9 (2.7)	90.8 (2.1)	59.4 (6.5)	93.4 (2.7)
Male (%)	24 (27.6)	108 (33.0)	834 (44.9)	785 (45.1)	199 (30.4)	111 (26.2)	747 (46.4)	350 (37.6)
Body mass index (kg/m^2)	24.5 (3.3)	25.8 (3.8)	26.7 (3.9)	26.9 (3.9)	26.6 (4.0)	25.9 (4.4)	25.3 (3.6)	25.5 (3.6)
Total cholesterol (mmol/l)	5.32 (1.02)	5.65 (1.08)	5.85 (1.00)	5.77 (0.98)	5.77 (1.09)	5.22 (1.04)	5.59 (1.12)	NA
LDL cholesterol (mmol/l)	3.11 (0.82)	3.37 (0.91)	3.74 (0.92)	3.69 (0.87)	3.67 (1.00)	3.26 (0.89)	3.32 (0.93)	NA
HDL cholesterol (mmol/l)	1.46 (0.44)	1.46 (0.49)	1.38 (0.39)	1.38 (0.40)	1.43 (0.44)	1.36 (0.42)	1.45 (0.44)	NA
Triglycerides (mmol/l)	1.74 (1.19)	1.87 (1.21)	1.62 (0.96)	1.53 (0.77)	1.50 (0.70)	1.42 (0.87)	1.76 (1.03)	NA
Systolic blood pressure (mmHg)	128.3 (13.9)	138.7 (20.4)	140.6 (20.1)	146.4 (21.7)	147.7 (21.6)	148.0 (19.3)	142.1 (19.9)	NA
Cohort (%)								
LLS Partners	87 (100)	327 (100)	284 (15.3)	42 (2.4)	0 (0)	0 (0)		
Leiden 85-plus study	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	316 (74.6)		
Rotterdam Study	0 (0)	0 (0)	1575 (84.7)	1697 (97.6)	655 (100)	108 (25.4)		

Continuous variables are presented as means and standard deviations, categorical variables are presented as numbers and percentages.
NA, not available.
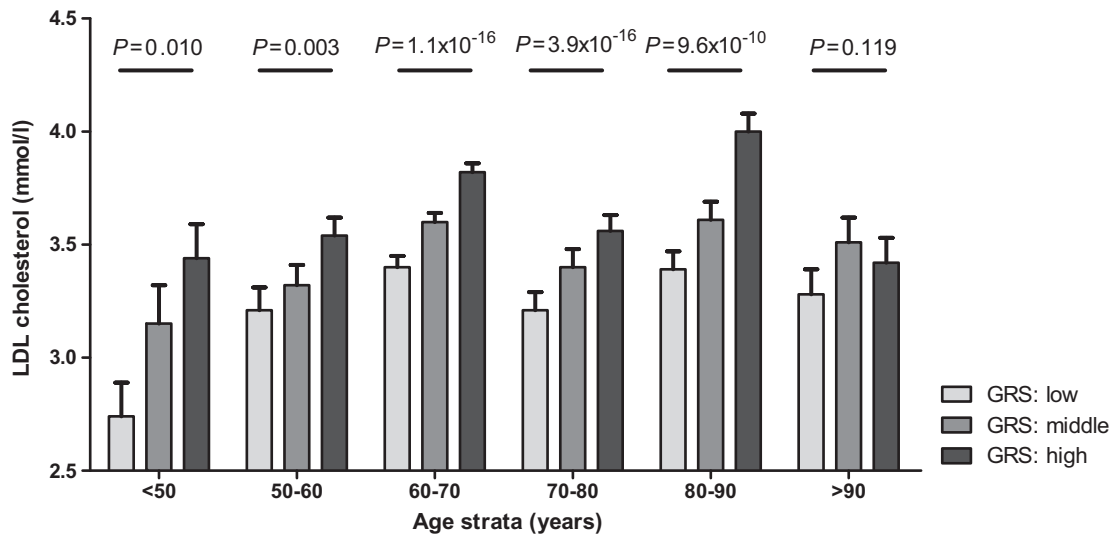
Figure 2. LDL cholesterol levels per GRS category in different age strata, using individual-level data of LLS partners, Leiden 85-plus study and the Rotterdam Study. Means and standard errors (SEs) were assessed using a general linear model adjusted for age, sex and cohort. P-values were assessed using the continuous values of the LDL GRS.

and chronological age [$\beta = -0.21$ (SE = 0.09) per 10-year increase in age, $P = 0.018$, adjusted for sex and cohort]. Additional separate analyses were performed in participants aged ≥50 years and in participants aged ≥70 years. In both age groups, the significant association between the LDL GRS and age remained when excluding the younger participants [≥50 years: $\beta = -0.21$ (SE = 0.09) per 10-year increase in age, $P = 0.023$; ≥70 years: $\beta = -0.46$ (SE = 0.16) per 10-year increase in age, $P = 0.004$]. Additional adjustment for HDL-C and triglycerides did not change any of the analyses.
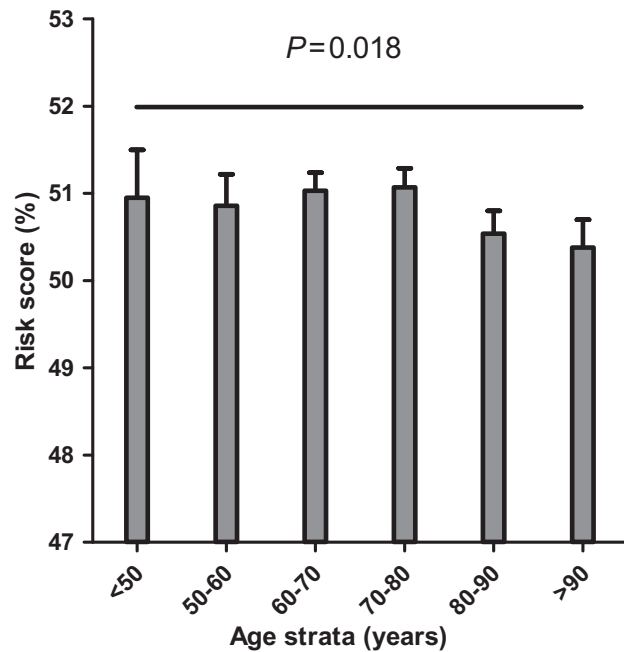
Next, we investigated whether the LDL GRS was associated with familial longevity. For this purpose, we compared the mean LDL GRS in the LLS nonagenarians vs the individuals aged ≥90 years from the Leiden 85-plus study and the Rotterdam Study (Figure 4A), and in the LLS offspring vs the LLS partners (Figure 4B). The mean GRS was significantly lower for individuals with a predisposition for longevity compared with the individuals from the general population within the same age range. LLS nonagenarians had a mean LDL GRS of 49.8% (SD = 0.2) compared with a mean LDL GRS of 50.5% (SE = 0.3) for the individuals aged ≥90 years from the general population ($P_{\text{difference}} = 0.029$). LLS offspring had a mean LDL GRS of 50.0% (SE = 0.2), whereas the partners had a mean LDL GRS of 50.7% (SE = 0.2) ($P_{\text{difference}} = 0.005$). Additional

adjustment for HDL-C and triglycerides did not change the association between LLS offspring and partners ($P = 0.008$).

Finally, we investigated the association between the LDL GRS and mortality in the elderly. For this purpose, we used the data of the LLS nonagenarians and of participants aged ≥ 90 years from the Leiden 85-plus study and the Rotterdam Study. The combined analysis of the three studies showed a significant association between the LDL
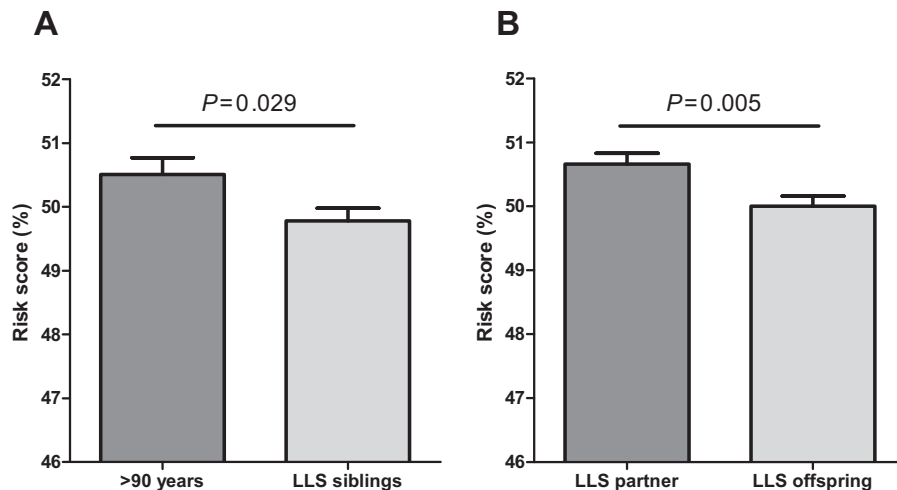
GRS and increased all-cause mortality (Table 2). Individuals in the middle LDL GRS group had an incidence rate ratio (IRR) of 1.08, [95% confidence interval (CI): 0.96-1.22], and individuals in the highest group had an IRR of 1.13 (95% CI: 1.00-1.26), compared with individuals in the lowest LDL GRS group (P-trend = 0.008). Analyses in the individual studies showed a significant association between LDL GRS and mortality in the LLS nonagenarians ($P = 0.008$), with 23% increased mortality risk (95% CI: 1.06-1.44) for individuals in the highest LDL GRS group, compared with individuals in the lowest group. Within the Leiden 85-plus study and the Rotterdam Study, the LDL GRS was not significantly associated with mortality ($P = 0.467$ and $P = 0.987$, respectively).

Discussion

In old age, the importance of high LDL-C levels as risk factor for mortality is unclear since observational studies have shown no or inverse associations. Due to confounding or reversed causality, the plasma LDL-C levels may not reflect the lifetime level. To overcome the potential influences of reverse causality and confounding, we used in the current study the LDL GRS as an instrumental variable. The LDL GRS was strongly associated with LDL-C levels and the number of LDL-increasing alleles decreased with increasing age. Furthermore, individuals with a genetic predisposition for longevity had a lower LDL GRS compared with age-matched controls. Finally, we showed that the LDL GRS was associated with all-cause mortality at ages above 90 years in the pooled analysis of three independent populations, although this effect was mainly driven by one study. All these results indicate that a genetic

Figure 3. Mean percentage of LDL GRS for each age category, including LLS partners, participants of the Leiden 85-plus study and the Rotterdam Study. The LDL GRS is plotted as mean percentage with standard error. *P*-value is calculated for the association between age and LDL GRS, adjusted for sex and cohort.

Figure 4. Mean percentage of LDL GRS in participants of the general population (dark grey) and genetically enriched for longevity (light grey). (A) Subjects aged ≥ 90 years from the Leiden 85-plus study and Rotterdam Study vs Leiden Longevity study (LLS) nonagenarians;.(B) LLS partners vs offspring. GRS is plotted as mean with standard error, adjusted for age, sex and familial relations.

Table 2. Association between LDL genetic risk score categories and mortality in participants aged ≥90 years

Study	Genetic risk score category									IRR (95% CI)		P-value
	Low			Middle			High			2 vs 1	3 vs 1	
	Deaths	PY (x1000)	IR (per 1000)	Deaths	PY (x1000)	IR (per 1000)	Deaths	PY (x1000)	IR (per 1000)			
Leiden 85-plus study	72	0.605	119.04 (94.49–149.97)	80	0.663	120.74 (96.98–150.32)	118	1.049	112.49 (93.92–134.74)	1.02 (0.84–1.23)	0.94 (0.78–1.13)	0.467
Rotterdam Study	34	0.362	93.97 (67.14–131.51)	36	0.372	96.70 (69.75–134.06)	38	0.404	94.05 (68.43–129.25)	1.02 (0.91–1.15)	1.00 (0.88–1.15)	0.987
LLS nonagenarians	262	1.157	226.51 (200.68–255.66)	282	1.138	247.75 (220.45–278.42)	248	0.936	264.95 (233.94–300.06)	1.11 (0.95–1.29)	1.23 (1.06–1.44)	0.008
Combined	368	2.123	173.31 (156.48–191.95)	398	2.173	183.15 (166.01–202.05)	404	2.389	169.11 (153.39–186.43)	1.08 (0.96–1.22)	1.13 (1.00–1.26)	0.043

Incidence rates and incidence rate ratios are shown with their 95% confidence interval. Incidence rate ratios were assessed using Poisson analysis, adjusted for age, sex, cohort and, where necessary, familial relations. *P*-values were assessed using the continuous values of the LDL GRS.

APY, person years; IR, incidence rate; IRR, incidence rate ratio; CI, confidence interval.

predisposition to high LDL-C levels contributes to mortality throughout life, including in the oldest old, and a beneficial LDL genetic profile is associated with familial longevity.

Observational studies have repeatedly shown a positive association between high cholesterol levels and increased mortality risks.[18] However, it is unclear whether this positive association remains in the elderly. Several studies in people aged 80 years and over showed an association between low total cholesterol levels and increased mortality.[5] Previously reported analysis of the Leiden 85-plus study did not observe any association between high LDL-C levels and mortality, and high total cholesterol levels were associated with longevity.[3,4] Cholesterol levels of elderly aged 85 years and over might not reflect their lifetime cholesterol level, due to reverse causality and possible selective survival.[3] This might also be an explanation for the lack of association between the LDL GRS and LDL-C levels in the participants aged 90 years and older and in the Leiden 85-plus study. Our results using the LDL GRS as an instrumental variable indicate that the results from observational studies in elderly using plasma cholesterol as a reflection of risk were probably biased.

The observed association between the LDL GRS and mortality was only significant in the LLS siblings and the combined analysis. This might be explained by the lower number of individuals aged 90 years and over in the Leiden 85-plus and Rotterdam Study compared with the LLS siblings. In addition, in the Leiden 85-plus study 75% of the participants were deceased at the end of follow-up. This high number and the relative small sample size could have influenced the accuracy of the performed analysis. We did observe that the level of LDL GRS decreased with increasing age; this was, however, not reflected in all prospective studies. A similar phenomenon was observed earlier for the

APOE gene. A lower frequency of the APOE ε4 allele with increasing age had already been reported in 1988.[19] However, associations between the APOE gene and mortality have been reported since 1994 in large studies.[20,21] More recently, the association between genetic variation in the APOE gene and longevity has repeatedly been validated in large prospective studies with sufficient statistical power.[21,22] Therefore, our findings should also be validated in larger prospective studies from different countries.

Genetic risk scores based on SNPs associated with cholesterol levels have been used previously. Within the CARDIoGRAM consortium, including more than 53 146 myocardial infarction cases and controls, the association between cholesterol levels and the risk of myocardial infarction (MI) was compared with the association between GRS and the risk of MI.[23] An increase in both plasma LDL-C levels and LDL-C conferred by the GRS was associated with an increased risk of MI. Increased HDL cholesterol was associated with a decreased risk for MI, although the HDL GRS was not associated with the risk for MI, indicating that HDL cholesterol is not a causal risk factor for MI.[23] Recently, a GRS based on LDL-C SNPs was tested in two British prospective studies, including middle-aged men and women.[24] Participants in the top quintile of the genetic score distribution tended to have a 36–49% increased risk of having a high cardiovascular disease (CVD) risk, determined by the Framingham 10-year CVD risk as more than 20%, compared with individuals in the lowest quintile. Our study shows that an association with mortality is still present at old age.

To create our LDL GRS, we used all SNPs associated with LDL-C cholesterol in the most recent analysis of the Global Lipids Genetics Consortium.[8] Some of the SNPs associated with LDL-C are also associated with total cholesterol, HDL-C or triglyceride levels. These associations could

possibly lead to pleiotropic effects of the GRS. Two epidemiological approaches to remove possible pleotropic effects are widely used.[25] The first approach is by using the residuals of the regression analysis between the target phenotype and the possible pleiotropic phenotypes, as was previously described by Do *et al.*[26] The other approach is to test whether the SNP or GRS is associated with the target phenotype after adjusting for the other phenotypes.[25] Both approaches to remove pleiotropic effects might have limitations.[25] To overcome the potential pleiotropic effects in our study, the analyses regarding the association with LDL-C levels and age were additionally adjusted for HDL-C and triglyceride levels, which did not affect the observed associations (Supplementary Figures 2 and 3, available as Supplementary data at *IJE* online). Hereby we reduced the possibility of the presence of pleiotropic effects in our analyses. However, we cannot conclude that we have completely removed all possible pleiotropic effects. In addition, LDL-C-associated SNPs might also be associated with other traits such as body mass index (BMI) or blood pressure.[8] Additional adjustment for BMI and systolic blood pressure also did not influence our findings (data not shown).

In the current study, we observed a difference in LDL GRS between offspring of nonagenarians and their spouses and between LLS nonagenarians and individuals aged ≥90 years. Individuals with a genetic predisposition for longevity had a lower LDL GRS, indicating the beneficial effects of low LDL cholesterol levels. This finding is the first difference in genetic risk scores observed between the LLS offspring and partners. Previous studies found a lower prevalence of diabetes mellitus, hypertension and myocardial infarction in LLS offspring compared with their partners.[27] Furthermore, the offspring had a more beneficial metabolic profile.[28] A GRS based on diabetes risk alleles has previously been tested in the LLS partners and offspring and, despite the better glucose tolerance of the offspring, this was not associated with differences in GRS.[28]

Novel platforms, such as proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, are used for the identification and quantification of a large number of low-molecular-weight metabolites in the blood. A previous study showed that ^{1}H-NMR metabolites show an improved prediction of all-cause mortality risk above established risk factors,[29] whereas another study showed that a score based on such metabolites associates with cardiovascular disease risk independently of LDL-C levels.[30] The same metabolite profiles are subjected to GWAS studies to find the genetic variation associated with these metabolites.[31,32] This will allow Mendelian randomization studies of small metabolites relevant for cardiovascular disease in the near future.

To summarize, previous observational studies including older individuals have shown no or inverse associations between cholesterol levels and mortality, suggesting that the causal relation between LDL and (cardiovascular) disease is absent at old age. Results of the current study indicate that a genetic predisposition to high LDL-C contributes to mortality throughout life, including in the oldest old, and a beneficial LDL genetic risk profile is associated with familial longevity.

Supplementary Data

Supplementary data are available at *IJE* online.

Author contributions

I.P., J.D., S.T., A.J.M.C., P.E.S., R.G.J.W., O.H.F., A.H. and J.W.J. were involved in the design of the current study. I.P., J.D., S.S., A.D. and S.T. were involved in data collection and data analysis. I.P., J.D., S.T., A.J.M.C., P.E.S., R.G.J.W. and J.W.J. were involved in data interpretation. I.P. and S.T. were involved in writing the manuscript. I.P., J.D., S.S., S.T., A.J.M.C., B.T.H., O.H.F., A.H., A.D., P.E.S., R.G.J.W. and JWJ were involved in critical review and final approval of the report.

Conflict of interest: We declare that we have no conflicts of interest.

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