Low LDL cholesterol, PCSK9 and HMGCR genetic variation, and risk of Alzheimer's disease and Parkinson's disease
Mendelian randomisation study

Benn, Marianne; Nordestgaard, Børge G.; Frikke-Schmidt, Ruth; Tybjærg-Hansen, Anne

Low LDL cholesterol, *PCSK9* and *HMGCR* genetic variation, and risk of Alzheimer's disease and Parkinson's disease: Mendelian randomisation study

Marianne Benn,[1,2,3] Børge G Nordestgaard,[2,3,4,5] Ruth Frikke-Schmidt,[1,2,3,5] Anne Tybjærg-Hansen[1,2,3,5]

[1]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 3, 2100 Copenhagen, Denmark

[2]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark

[3]Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

[4]Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark

[5]The Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Denmark

Correspondence to: M Benn Marianne.benn@regionh.dk
Additional material is published online only. To view please visit the journal online.

ABSTRACT

OBJECTIVE
To test the hypothesis that low density lipoprotein (LDL) cholesterol due to genetic variation in the genes responsible for LDL cholesterol metabolism and biosynthesis(*PCSK9* and 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), respectively) is associated with a high risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease in the general population.

DESIGN
Mendelian randomisation study.

SETTING
Copenhagen General Population Study and Copenhagen City Heart Study.

PARTICIPANTS
111 194 individuals from the Danish general population.

MAIN OUTCOME MEASURES
Risk of Alzheimer's disease, vascular dementia, all dementia, and Parkinson's disease.

RESULTS
In observational analyses, the multifactorially adjusted hazard ratio for Parkinson's disease in participants with an LDL cholesterol level <1.8 mmol/L versus ≥4.0 mmol/L was 1.70 (95% confidence interval 1.03 to 2.79), whereas the corresponding hazard ratios for Alzheimer's disease, vascular dementia, or any dementia did not differ from 1.0. *PCSK9* and *HMGCR* variants combined were associated with a 9.3% lower LDL cholesterol level. In genetic, causal analyses adjusted for age, sex, and year of birth, the risk ratios for a lifelong 1 mmol/L lower LDL cholesterol level were 0.57 (0.27 to 1.17) for Alzheimer's disease, 0.81 (0.34 to 1.89) for vascular dementia, 0.66 (0.34 to 1.26) for any dementia, and 1.02 (0.26 to 4.00) for Parkinson's disease. Summary level data from the International Genomics of Alzheimer's Project using Egger Mendelian randomisation analysis gave a risk ratio for Alzheimer's disease of 0.24 (0.02 to 2.79) for 26 *PCSK9* and *HMGCR* variants, and of 0.64 (0.52 to 0.79) for 380 variants of LDL cholesterol level lowering.

CONCLUSION
Low LDL cholesterol levels due to *PCSK9* and *HMGCR* variants had no causal effect on high risk of Alzheimer's disease, vascular dementia, any dementia, or Parkinson's disease; however, low LDL cholesterol levels may have a causal effect in reducing the risk of Alzheimer's disease.

Introduction

Inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase with statins and inhibiting proprotein convertase subtilisin-kexin type 9 (PCSK9) with monoclonal antibodies lower low density lipoprotein (LDL) cholesterol levels, and statins also lower the risk of cardiovascular disease.[1-6] However, cholesterol is a major constituent of the myelin encircling neurons in the brain, and the risk of neurological diseases such as Alzheimer's disease and Parkinson's disease have been reported in people treated with traditional cholesterol lowering drugs,[7] in particular statins,[8 9] although results have been conflicting.[10-13] Also, early results on PCSK9 inhibitors have suggested that compared with placebo they might increase the frequency of neurological symptoms (evolocumab 0.9% *v* 0.3% and alirocumab 1.2% *v* 0.5%), although these results were not statistically significant.[5 6 14] Thus it is not known whether a low LDL cholesterol level in itself has a causal effect on risk of Alzheimer's disease and Parkinson's disease. This is important, as current guidelines on the treatment and prevention of cardiovascular disease recommend an LDL cholesterol level of less than 1.8 mmol/L in patients at very high risk of cardiovascular disease and less than 2.6 mmol/L in patients at high risk.[15]

To investigate whether a low LDL cholesterol level has a causal effect on risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease, we used the Mendelian randomisation approach with variants in the genes encoding HMG-CoA reductase and PCSK9, the drug targets of statins and PCSK9 inhibitors.[16 17] Mendelian randomisation is an epidemiological approach that aims to circumvent confounding and reverse causation by use of genetic variation in populations.[18 19] Because of the random assortment of genetic variants at conception, variants with an effect on a modifiable exposure of interest (that is, LDL

cholesterol) are randomly distributed in relation to most potential confounders. Also, because genetic variants are determined at conception and remain constant throughout life, the Mendelian randomisation design is not influenced by reverse causation. Thus, if a low LDL cholesterol level has a causal effect on risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease, genetic variants that lower LDL cholesterol levels lifelong would be expected to also increase the risk of disease.

Using a Mendelian randomisation design in 111 194 individuals from two prospective general population studies, the Copenhagen General Population Study and the Copenhagen City Heart Study, we tested the hypothesis that a low LDL cholesterol level due to genetic variation in *PCSK9* and *HMGCR* is associated with a high risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease. We tested firstly whether LDL cholesterol level at baseline is associated prospectively with disease (see study design supplementary figure); secondly, whether scores of LDL cholesterol lowering alleles of *PCSK9* R46L (rs11591147), R237W (rs148195424), I474V (rs562556), and E670G (rs505151), and *HMGCR* (rs17238484) are associated with low LDL cholesterol concentrations as expected; thirdly, whether LDL cholesterol lowering alleles are associated directly with risk of diseases, as an indication of a causal effect of low LDL cholesterol levels on risk of disease; and fourthly, whether the causal effect of low LDL cholesterol levels is consistent with the corresponding observational associations using instrumental variable analysis. For Alzheimer's disease, we also included a risk estimate using summary level data from the Global Lipid Genetics Consortium[20] and the International Genomics of Alzheimer's Project[21] on *PCSK9* and *HMGCR* genetic variants combined, and finally to increase statistical power on all LDL cholesterol lowering variants included in the International Genomics of Alzheimer's Project study. The *PCSK9* and *HMGCR* studies were the primary hypotheses and the generic score of all LDL cholesterol lowering variants used in Global Lipid Genetics Consortium/International Genomics of Alzheimer's Project following a negative result for *PCSK9* and *HMGCR*.

Methods
Participants
We included participants from two similar prospective studies of the Danish general population: the Copenhagen General Population Study (n=99 993) and the Copenhagen City Heart Study (n=11 201).[22 24-25] Combining these two studies yielded a total of 111 194 participants of whom 1001 developed Alzheimer's disease, 256 vascular dementia, 2154 any dementia, and 460 Parkinson's disease during up to 37 years of follow-up (median 8.2 years). All participants were white and of Danish descent, and none were included in more than one study. No participants were lost to follow-up.

The Copenhagen General Population Study
The Copenhagen General Population Study was initiated in 2003 and enrolment is ongoing.[22 24-26]

Participants were selected based on the national Danish civil registration system to reflect the adult white Danish population at age 20 to 100 years. Data were obtained from a questionnaire, reviewed together with an investigator at the day of attendance, a physical examination, and from blood samples, including DNA extraction.

The Copenhagen City Heart Study
The Copenhagen City Heart Study[22 24 26] is a prospective study of the Danish general population initiated in 1976-78, with follow-up examinations in 1981-83, 1991-94, and 2001-03. Participants were recruited and examined exactly as in the Copenhagen General Population Study. Baseline was considered the first examination an individual received in either 1991-94 or 2001-03, and where LDL cholesterol was measured.

Patient involvement
No patients were involved in the design of the study, recruitment, or conduct of the study. The outcome measurements were based on public discussion on the topic and not directly on informed participant priorities, experiences, or preferences. Results will, after scientific publication, be disseminated to the public in general.

Endpoints
Diagnoses of endpoints according to the World Health Organization ICD-8 and ICD-10 codes (international classification of diseases, eighth and 10th revisions, respectively) were collected from 1977 to 10 November 2014 by reviewing all hospital admissions and diagnoses in the national Danish patient registry and all causes of death in the national Danish causes of death registry.[27 28] Endpoints were defined as: Alzheimer's disease (ICD-8: 290.10; ICD-10: F00, G30), vascular dementia (ICD-10: F01), any dementia—that is, Alzheimer's disease, vascular dementia, and non-specified dementia combined (ICD-8: 290; ICD10: F00, F01, F03, G30),[27 28] and Parkinson's disease (ICD-8: 342; ICD10: G20-G22). In Denmark these diseases are diagnosed by specialists in neurology, and a validation with full clinical investigation of the register based dementia diagnoses has previously been performed, with diagnostic validity of the Danish hospital registries considered to be high.[29] The validity of the register based dementia diagnoses was further ensured by the presence of the well known association with the apolipoprotein ε4 allele in the Copenhagen General Population Study.[28]

Follow-up began at the first inclusion into a study and ended with censoring at the date of death, occurrence of an event, emigration (n=811), or on 10 November 2014 (corresponding to the end of follow-up for the least updated register), whichever came first.

LDL cholesterol and PCSK9
The LDL cholesterol level was calculated using the Friedewald equation if plasma triglyceride levels were ≤4.0 mmol/L and measured by a direct enzymatic method at higher triglyceride concentrations (Thermo Fisher Scientific/Konelab). For the observational classification,

we multiplied the plasma LDL cholesterol concentrations by 1.43 in those using cholesterol lowering drugs, corresponding to an estimated 30% reduction in LDL cholesterol level.[30] Use of cholesterol lowering drugs was self reported, with more than 97% accounted for by statins.

BG Medicine (Waltham, MA) measured plasma PCSK9 levels in a subset of participants with a sandwich enzyme linked immunosorbent assay using antibodies supplied by Merck (Whitehouse Station, NJ). The intra-assay and inter-assay coefficients of variation were 6.8% and 12.5%, respectively.

Covariates
Plasma levels of total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were measured using standard hospital assays. Hypertension was a systolic blood pressure ≥140 mm Hg (≥135 mm Hg for participants with diabetes), diastolic blood pressure ≥90 mm Hg (≥85 mm Hg for participants with diabetes), and/or the participant used antihypertensive drugs prescribed specifically for hypertension.[31] Participants also reported on smoking, amount smoked daily, and ages of starting and quitting, and we used this information to calculate pack years smoked. We coded work and leisure time physical activity as "low" for 0-2 hours moderate activity, "intermediate" for 2-4 hours activity, and "high" for more than four hours moderate or vigorous activity each week during either work or leisure time. Information on alcohol consumption was summarised in units weekly (1 unit about 12 g of alcohol). Education was recorded as <10, 10 to 12, or ≥13 years of completed education. For women, menopausal status was also recorded. Missing data on covariates varied from 0 to 1% for any individual variable. In observational analyses we excluded participants with missing covariates on an analysis basis (<1.5% excluded). Participants included in genetic analyses had complete data on age, sex, genotypes, and endpoints.

Genotyping
An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) and TaqMan based assays were used to genotype for LDL cholesterol associated genotypes—that is, PCSK9 R46L (rs11591147), R237W (rs148195424), I474V (rs562556), E670G (rs505151), and HMGCR (rs17238484). We constructed a PCSK9 allele score by summation of number of LDL cholesterol lowering alleles; we ordered the HMGCR genotype by LDL cholesterol concentration; and the combined genotype was the sum of PCSK9 and HMCGR LDL cholesterol lowering alleles. Because heterozygosity or homozygosity for the common E670G (rs505151) genotype raises LDL cholesterol levels, whereas the R46L (rs11591147), R237W (rs148195424), and I474V (rs562556) genotypes lowers LDL cholesterol levels compared with the population mean, we coded the allele score −1 for each LDL cholesterol raising allele and 1 for each LDL cholesterol lowering allele, resulting in a score of −1, 0 (participants being non-carriers for all four PCSK9 genotypes), 1, and 2-4 LDL cholesterol

lowering alleles. Some allele groups were combined to ensure categories included a sufficient number of participants. We used an unweighted score because most readers intuitively understand a simple summation of alleles; however, as a sensitivity analysis, we also generated weighted allele scoresfor PCSK9 genotypes and PCSK9 and HMGCR genotypes combined.

Statistical analyses
Data were analysed using Stata SE 13.1.[32] For genotypes, we tested a deviation from Hardy-Weinberg equilibrium using a Pearson χ^2 test. Linear regression examined the association between LDL cholesterol and PCSK9 plasma levels. This is reported as P for trend. Firstly, to test whether a low LDL cholesterol level was associated with risk of disease, we categorised participants by baseline LDL cholesterol concentrations into categories reflecting values recommended for lipid lowering treatment (<1.8 mmol/L, 1.8-2.59 mmol/L, 2.60-3.99 mmol/L) compared with a large reference group (≥4.0 mmol/L).[15] As a sensitivity analysis, we also examined this association excluding participants using lipid lowering drugs and categorising LDL cholesterol levels into fifths. We tested the associations between low LDL cholesterol level and diseases using Cox regression models adjusted for age, sex, year of birth, hypertension, smoking, physical activity, alcohol consumption, education, and, for women, menopausal status. Adjustment for year of birth was done to accommodate changes in diagnostic criteria and treatment over calendar time. To test for trends for statistical significance across ordered categories of low LDL cholesterol levels and genotypes we used the non-parametric Cuzick's extension of a Wilcoxon rank sum test.

Secondly, we used linear regression to test whether genotypes and allele scores were associated with low LDL cholesterol levels. Because the distribution of LDL cholesterol in the population is slightly skewed towards higher concentrations, we performed all analyses on log transformed values of LDL cholesterol. For genotypes to be used as unconfounded instruments in the Mendelian randomisation approach, they should not be associated with known confounders for disease. To test this, we used logistic regression to assess whether observational low LDL cholesterol levels or the genotypes and allele scores were associated with the potential confounders of age, sex, hypertension, smoking, physical activity, alcohol consumption, education, and, for women, menopausal status.

Thirdly, to test whether genotypes and allele scores were associated with risk of disease we used Cox regression models adjusted for age, sex, and year of birth.

Fourthly, because genotype is constant throughout life, and hence impervious to reverse causation, we carried out instrumental variable analysis to assess the potential causal effect of genetically low LDL cholesterol levels on risk of disease using the user written ivreg2 and ivpois commands in Stata.[33][34]ivpois implements generalised methods of moment conditions equivalent to the multiplicative structural mean model using instrumental variables,[34] with predicted

values of the mean of LDL cholesterol level within each genotype score category.[35][36] Strength of the instruments (that is, the strength of the association of the genotypes with LDL cholesterol level) was confirmed by F statistics of more than 73 (F>10 is considered acceptable) from regressions using *ivreg*2.[18] Power to exclude a causal risk ratio of disease at a two sided α of 0.05 and β of 80% was calculated with an online power calculation tool (https://sb452.shinyapps.io/power/) for instrumental variable analysis in Mendelian randomisation studies with binary outcomes using the ratio (or Wald) method.[37]

Finally, we conducted summary level Mendelian randomisation analyses,[38] firstly using genetic variants in *PCSK9* and *HMGCR* combined and secondly using all LDL cholesterol lowering genetic variants identified in the published genome-wide association study (GWAS) Global Lipid Genetics Consortium (n=95 454),[20] except variants in the *APOE* gene with numerous well known pleotropic effects, and for each LDL cholesterol lowering variant estimated risk of Alzheimer's disease, using the International Genomics of Alzheimer's Project (n=17 008 Alzheimer's disease cases and n=37 154 controls).[21] The second part using all available genetic variants was done to increase the generalisability of the study for pathways examined, and because none of the *PCSK9* and *HMGCR* variants in the Global Lipid Genetics Consortium/International Genomics of Alzheimer's Project were associated strongly with LDL cholesterol concentrations (see appendix A for information on these study populations). In brief, the genetic variants were selected from the Global Lipid Genetics Consortium to either be located in the *PCSK9* (chromosome 1: 55 039 548-55 064 852) or in the *HMGCR* (chromosome 5: 75 338 133-75 344 300) genes or to be associated with low LDL cholesterol levels with P values of <1E-7 (960 variants). The threshold of <1E-7 was selected to ensure a high degree of independence of association between the genetic variants and low density lipoprotein cholesterol, even with multiple testing.[39] In both samples we excluded variants with ambiguous information on minor allele, effect allele, and direction of the association. Also, we excluded variants with ambiguous direction of the association with LDL cholesterol level among laboratories contributing to the Global Lipid Genetics Consortium and variants with missing values. We then pruned the variants manually for pleiotropy excluding variants with reported associations with confounding phenotypes using GWAS Central (www.gwascentral.org/); and for linkage disequilibrium using the SNP Annotation and Proxy search (SNAP, www.broadinstitute.org/mpg/snap/), excluding variants in linkage disequilibrium with an R^2>0.80, leaving 26 variants from the *PCSK9* and *HMGCR* genes (see supplementary table 1), and 380 variants using the wider selection criteria (see supplementary table 2). To obtain robust summary estimates of the causal effect of low LDL cholesterol level on risk of Alzheimer's disease from the summary level data, we performed regression analysis using inverse variance weighting and Egger Mendelian randomisation using the user written *mregger* command in

Stata,[40] where the inverse variance weighted estimates correspond to those obtained using conventional Mendelian randomisation on individual level data, and Egger Mendelian randomisation analysis to estimate a causal effect accounting for direct pleiotropic effects of the variants, and finally weighted median of instrumental variable estimates using the user written *mrmedian* command in Stata[41] accounting for up to 50% of information coming from invalid or weak instruments.[40][41]

Results
Of 111 194 participants 4087 (3.7%) had LDL cholesterol levels of <1.8 mmol/L, 22 335 (18%) of 1.8-2.59 mmol/L, 57 847 (52%) of 2.6-3.99 mmol/L, and 28 925 (26%) of ≥4.0 mmol/L. During follow-up, 1001 participants developed Alzheimer's disease, 256 vascular dementia, 2154 any dementia, and 460 Parkinson's disease. Participants with an LDL cholesterol concentration <1.8 mmol/L versus ≥4.0 mmol/L were younger, had higher HDL cholesterol and lower triglyceride concentrations, and were more likely to have been in education for more than 13 years (table 1). Also, women were more likely to be premenopausal. Plasma LDL cholesterol levels were directly associated with plasma PCSK9 concentrations (P for trend=0.004) and showed a slightly skewed distribution in the population, with a tail towards higher levels (fig 1). *PCSK9* R46L (rs11591147), R237W (rs148195424), I474V (rs562556), E670G (rs505151), and *HMGCR* (rs17238484) genotype distributions did not deviate from Hardy-Weinberg expectations (all P>0.05).

LDL cholesterol and risk of disease: observational estimates
Observationally, categories of lower LDL cholesterol levels reflecting values recommended for lipid lowering treatment (<1.8 mmol/L, 1.8-2.59 mmol/L, 2.6-3.99 mmol/L) were associated with a stepwise higher risk of Parkinson's disease (P for trend=0.009; Bonferroni corrected for multiple testing P for trend=0.036), but not of Alzheimer's disease, vascular dementia, or any dementia (fig 2). During a median follow-up of 8.2 years (range 0 to 37), the multifactorially adjusted hazard ratio for Parkinson's disease in those with an LDL cholesterol level of <1.8 mmol/L versus ≥4.0 mmol/L was 1.70 (95% confidence interval 1.03 to 2.79), whereas the corresponding hazard ratios for Alzheimer's disease, vascular dementia, or any dementia did not differ from 1.0. These results were similar when participants were categorised into fifths of LDL cholesterol levels (see supplementary figure 1), and when those using lipid lowering drugs were excluded (see supplementary figure 2). The association between LDL cholesterol level and risk of Parkinson's disease was not linear and risk was only increased at LDL cholesterol levels below 4 mmol/L (see supplementary figure 3).

Genotypes and plasma LDL cholesterol
An increasing number of *PCSK9* and *HMGCR* alleles separately and combined were associated with stepwise lower mean LDL cholesterol levels (fig 3, left panel). Scores of 2-4 versus –1 for *PCSK9* LDL cholesterol

Table 1 | Baseline characteristics of participants by low density lipoprotein (LDL) cholesterol level. Values are numbers (percentages) unless stated otherwise

Characteristics	LDL cholesterol (mmol/L)				P trend	All
	<1.8	1.8-2.59	2.6-3.99	≥4.0		
Participants	4087 (3.7)	20 335 (18)	57 847 (52)	28 925 (26)		111 194 (100)
Median (interquartile range) age (years)	49 (40-64)	51 (42-64)	56 (47-66)	58 (50-66)	<0.001	56 (46-66)
Women	2370 (58)	11 794 (58)	31 237 (54)	15 909 (55)	<0.001	61 310 (55)
Median (interquartile range) LDL cholesterol (mmol/L)	1.6 (1.3-1.7)	2.3 (2.1-2.5)	3.2 (2.9-3.6)	4.4 (4.1-4.8)	<0.001	3.2 (2.6-3.9)
Median (interquartile range) HDL cholesterol (mmol/L)	1.7 (1.3-2.1)	1.7 (1.3-2.1)	1.6 (1.3-2.0)	1.5 (1.2-1.8)	<0.001	1.6 (1.2-1.9)
Median (interquartile range) Triglycerides (mmol/L)	1.1 (0.7-1.8)	1.1 (0.8-1.7)	1.4 (1.0-2.0)	1.7 (1.2-2.4)	<0.001	1.4 (1.0-2.1)
Hypertension	695 (17)	2847 (14)	8677 (15)	5207 (18)	<0.001	17 426 (16)
Median (interquartile range) smoking, pack years (smokers only)	7.5 (0-27)	4.5 (0-23)	7.5 (0-27)	15 (0-30)	<0.001	9.5 (0-28)
Physical activity:						
Low	1717 (42)	8541 (42)	27 188 (47)	15 041 (52)	<0.001	52 487 (47)
Intermediate	1921 (47)	9761 (48)	26 031 (45)	11 859 (41)	0.003	49 572 (44)
High	450 (11)	2034 (10)	5206 (9)	2025 (7)	<0.001	9715 (8)
Median (interquartile range) alcohol consumption (units/week)*	6 (2-13)	6 (2-13)	6 (2-14)	6 (2-14)	0.14	6 (2-14)
Education (years):						
<10	858 (21)	4068 (20)	15040 (26)	9545 (33)	<0.001	29 511 (27)
10-13	2289 (56)	12 210 (60)	34 708 (60)	16 198 (56)	<0.001	65 405 (58)
≥13	940 (23)	4068 (20)	8099 (14)	3182 (11)	<0.001	16 289 (15)
Postmenopausal, women only	450 (19)	2713 (23)	9683 (31)	6205 (39)	<0.001	19 051 (31)

HDL=high density lipoprotein.
LDL cholesterol concentration was multiplied by 1.43 in participants receiving cholesterol lowering treatment, corresponding to an estimated 30% reduction in LDL cholesterol levels. Multiply by 38.6 to convert cholesterol values from mmol/L to mg/dL.
*1 unit alcohol is about 12 g.

lowering alleles was associated with 8.1% lower LDL cholesterol levels. The *PCSK9* alleles explained 2.7% of the variation in LDL cholesterol levels (F statistic=73). Scores of 2 versus 0 for *HMGCR* LDL cholesterol lowering alleles was associated with 3.0% lower cholesterol levels. The *HMGCR* alleles explained 0.2% of the variation in LDL cholesterol levels (F statistic=90). Scores of 4 versus –1 or 0 for *PCSK9* and *HMGCR* LDL cholesterol lowering alleles combined was associated with 9.3% lower LDL cholesterol levels. The combined allele score explained 1.7% of the variation in LDL cholesterol levels (F statistic=73).

Confounding factors
We tested whether potentially confounding factors were associated with LDL cholesterol level, disease, and genetic variants. Age, sex, hypertension, smoking, physical activity, alcohol consumption, educational level, and, for women menopausal status were all strongly associated with both low LDL cholesterol levels and/or with risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease (see supplementary figure 4), and may therefore confound the observational associations. However, the genotypes were not associated with any of the potential confounders, suggesting that pleiotropic effects through any of the above factors are not likely.

Genotypes and risk of disease: genetic estimates
Age, sex, and year of birth adjusted risks of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease were not statistically significantly different among participants scores of 2-4 *PCSK9* LDL cholesterol lowering alleles, 2 *HMGCR* LDL cholesterol lowering alleles, or 4 LDL cholesterol lowering alleles

combined compared with participants with scores of –1 or 0 LDL cholesterol lowering alleles (fig 4) (see supplementary table 3 for risk estimates). Using a weighted allele score for *PCSK9* and alleles combined gave similar results (see supplementary figure 5). Prevalence of dementia and Parkinson's disease is age dependent; however, stratifying analyses by below and above age 60 years gave similar results (see supplementary figure 6). *APOE* genotype (positive control) showed the known association of the ε43 and ε44 genotypes with risk of Alzheimer's disease, vascular dementia, and any dementia, confirming the validity of the endpoints used and study power to show positive associations.

Causal effect of low LDL cholesterol level on risk of disease
The hazard ratios for a 1 mmol/L lower observational LDL cholesterol level were 0.96 (0.91 to 1.02) for Alzheimer's disease, 1.09 (0.97 to 1.23) for vascular dementia, 1.01 (0.97 to 1.06) for any dementia, and 1.10 (1.00 to 1.21) for Parkinson's disease (fig 4). In genetics, causal analyses adjusted for age, sex, and year of birth, risk ratios for a 1 mmol/L lower LDL cholesterol level were 0.57 (95% confidence interval 0.27 to 1.17) for Alzheimer's disease, 0.81 (0.34 to 1.89) for vascular dementia, 0.66 (0.34 to 1.26) for any dementia, and 1.02 (0.26 to 4.00) for Parkinson's disease (fig 4). Using summary level data from the International Genomics of Alzheimer's Project for 26 variants in the *PCSK9* and *HMGCR* genes, the risk ratio for Alzheimer's disease was 2.04 (95% confidence interval 0.48 to 12) in an inverse variance weighted analysis, 0.24 (0.02 to 2.79) using Egger Mendelian randomisation analysis, and 4.66 (0.57 to 38.0) using a weighted median of instrumental variable estimates analysis. Corresponding estimates using 380
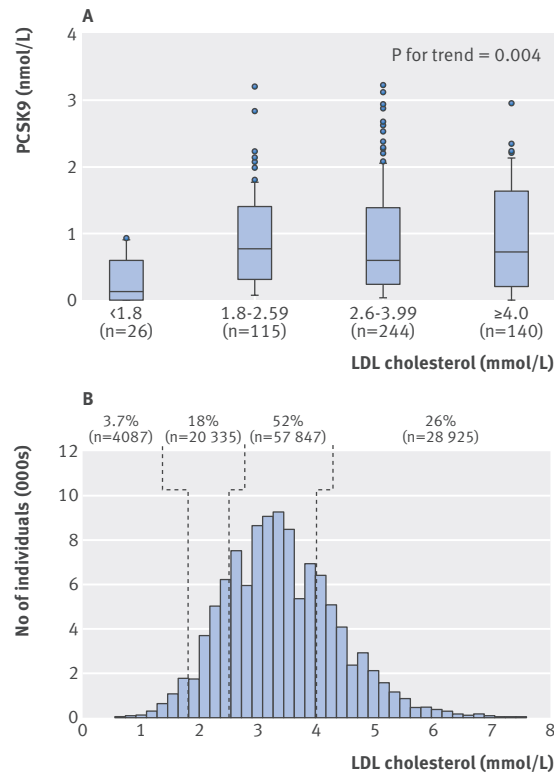
Fig 1 | (A) Plasma pro-protein convertase kexin-subtilisin 9 (PCSK9) concentration as a function of categories of low density lipoprotein (LDL) cholesterol in 489 participants from the Copenhagen General Population Study not receiving lipid lowering treatment. Bands inside boxes correspond to medians, bottoms and tops of the boxes to interquartile ranges, and whiskers to 10th and 90th centiles. P value is for trend across ordered groups from linear regression. N=number of participants. (B) Distribution of LDL cholesterol levels in general population and categories used in the study, reflecting values recommended for lipid lowering treatment (<1.8 mmol/L, 1.8-1.59 mmol/L, 2.6-3.99 mmol/L) compared with large reference group (≥4.0 mmol/L). N=number of participants in category and percentage of population

genetic variants associated with low LDL cholesterol levels according to the Global Lipid Genetics Consortium were 0.83 (0.75 to 0.92), 0.64 (0.52 to 0.79), and 0.97 (0.91 to 1.02), respectively. Data from the latter analyses showed a modest indication of directional pleiotropy for genetic variants with low precision, but this bias was not correlated with the association of genetic variants with LDL cholesterol level and the assumptions for using Egger regression were not violated (see supplementary figures 7 and 8).

Discussion

In 111 194 individuals from the general population, we found that low LDL cholesterol levels were associated observationally with a high risk of Parkinson's disease but not with Alzheimer's disease, vascular dementia, or any dementia. Low LDL cholesterol levels due to *PCSK9* and *HMGCR* genetic variants did not appear to increase the risk of Alzheimer's disease, vascular dementia, any dementia, or Parkinson's disease. However, using

similar data on 380 genetic variants associated with low LDL cholesterol levels, genetically low LDL cholesterol was associated with a reduced risk, suggesting a causal effect of low LDL cholesterol level in reducing the risk of Alzheimer's disease.

Strengths and weaknesses of this study

Strengths of the study include examination of a large number of individuals from a homogenous general population, access to individual participant data of a high validity, no losses to follow-up, and the use of the Mendelian randomisation approach. This approach allows us to examine potential causal effects of low LDL cholesterol on risk of disease, largely without confounding and definitely without reverse causation. Also, the inclusion of two sample Mendelian randomisation estimates from the Global Lipid Genetics Consortium and the International Genomics of Alzheimer's Project increases the generalisability of the results by adding risk estimates obtained from people of other ethnicities than white people and of low LDL cholesterol levels by more pathways than the *PCSK9* and *HMGCR*. Risk of Alzheimer's disease was estimated in data from the International Genomics of Alzheimer's Project using three summary data Mendelian randomisation analyses (fig 4): the conventional inverse variance weighted analysis (unadjusted for pleiotropy where a genetic variant is associated with more than one phenotype); an analysis using Egger Mendelian randomisation, which reduces over-estimation of a causal effect due to pleiotropy, provided the gene-exposure (ie, genetically low LDL cholesterol levels) does not correlate with the bias due to directional pleiotropy (the InSIDE assumption), but at the cost of lower power; and weighted median of instrumental variable estimates analysis accounting for up to 50% of information coming from invalid or weak instruments.[40][41] Risk estimates from these three analyses were all in the same direction, suggesting a causal effect of low LDL cholesterol levels in causing a lower risk of Alzheimer's disease.

A weakness is that the estimates obtained in the Copenhagen General Population Study and the Copenhagen City Heart Study rely on genetic variants from a few genes, which means that the causal estimates may not apply to mechanisms mediated by other genes; however, we carefully selected the genetic variants as those associated with the highest effect on LDL cholesterol levels to reduce risk of weak instruments. The current practice in Mendelian randomisation often involves a two sample Mendelian randomisation in which the causal effect is estimated as the weighted average of Wald estimator's for several genetic variants associated with the biomarker of interest—that is, low LDL cholesterol levels. It is often argued that this is especially powerful since current genome wide association study (GWAS) meta-analyses estimate very precisely the association between a particular genetic variant and the biomarker.[39] However, we question that this is true for our analyses. The GWAS estimates for the association between genotype and intermediate pheno-

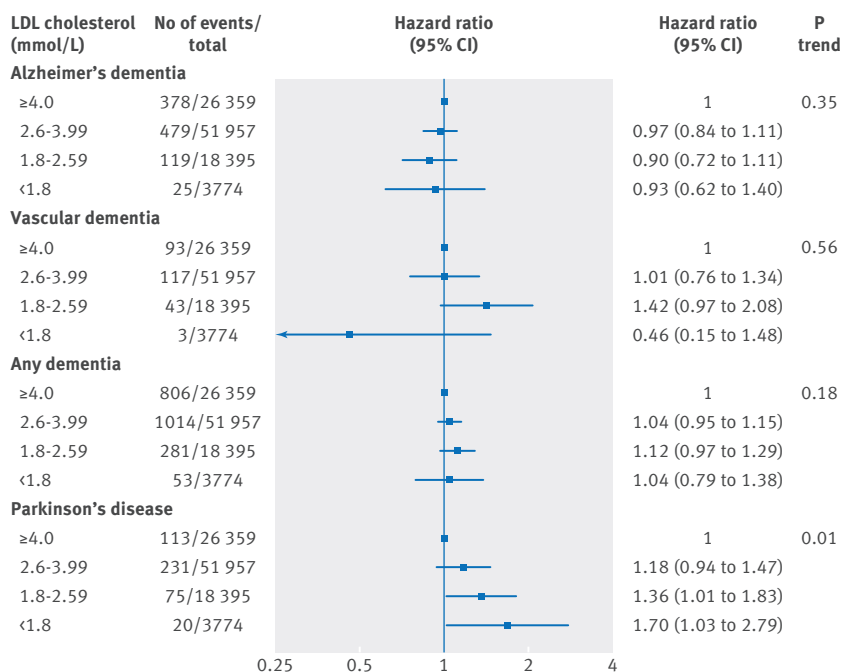| LDL cholesterol (mmol/L)	No of events/ total	Hazard ratio (95% CI)	Hazard ratio (95% CI)	P trend
Alzheimer's dementia				
≥4.0	378/26 359		1	0.35
2.6-3.99	479/51 957		0.97 (0.84 to 1.11)	
1.8-2.59	119/18 395		0.90 (0.72 to 1.11)	
<1.8	25/3774		0.93 (0.62 to 1.40)	
Vascular dementia				
≥4.0	93/26 359		1	0.56
2.6-3.99	117/51 957		1.01 (0.76 to 1.34)	
1.8-2.59	43/18 395		1.42 (0.97 to 2.08)	
<1.8	3/3774		0.46 (0.15 to 1.48)	
Any dementia				
≥4.0	806/26 359		1	0.18
2.6-3.99	1014/51 957		1.04 (0.95 to 1.15)	
1.8-2.59	281/18 395		1.12 (0.97 to 1.29)	
<1.8	53/3774		1.04 (0.79 to 1.38)	
Parkinson's disease				
≥4.0	113/26 359		1	0.01
2.6-3.99	231/51 957		1.18 (0.94 to 1.47)	
1.8-2.59	75/18 395		1.36 (1.01 to 1.83)	
<1.8	20/3774		1.70 (1.03 to 2.79)	

0.25   0.5   1   2   4

Fig 2 | Prospective risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease as a function of baseline low density lipoprotein (LDL) cholesterol in categories reflecting values recommended for lipid lowering treatment (<1.8 mmol/L, 1.8-1.59 mmol/L, 2.6-3.99 mmol/L) compared with a large reference group (≥4.0 mmol/L). Multifactorially adjusted for age, sex, birth year, smoking (pack years), alcohol (units/week), physical inactivity, income, education, and menopause for women. Individuals with an event before baseline were excluded. P values are for test for trend of hazard ratios across ordered groups. Total=number of participants

type are derived from many pooled cohorts where phenotype (ie, LDL cholesterol levels) often is measured using different methods in the various studies. Also, genetic variants in GWAS may not be the variants with the largest effect on the intermediate phenotype, as can be seen for *PCSK9* and *HMGCR* variants in the Global Lipid Genetics Consortium/International Genomics of Alzheimer's Project (see supplementary table 1). Furthermore, the genotype-phenotype association may differ among different ethnicities. In our study we used measurements performed at a single laboratory and we included around 110 000 white people of Danish descent to determine the genotype-phenotype association, and we used the same people to study the genotype-endpoint association. This might be a much more accurate determination than a pooled estimate from many different studies and ethnicities. However, the estimate obtained using data from the Global Lipid Genetics Consortium and International Genomics of Alzheimer's Project studies relies on several genes and ethnicities, and showed similar results, suggesting that a low LDL cholesterol level in itself is not associated with risk.

More cases and controls and thus more statistical power would have been ideal to better refute or confirm the hypothesis that low LDL cholesterol levels lead to a high risk of dementia and Parkinson's disease, particularly in the Copenhagen studies. As can be seen from figure 4, right column, the power to exclude a genetic association is limited for some of the endpoints. To balance this limitation, it is reassuring that within the same study population we showed the well known causal association between the *APOE* 4 allele and high risk of Alzheimer's disease. Furthermore, for Alzheimer's disease the inclusion of the Global Lipid Genetics Consortium and International Genomics of Alzheimer's Project studies vastly increased statistical power in the genetic studies. Nevertheless, the Copenhagen results are important, also because they provide the observational findings.

Observationally, there was a statistically significant trend for a higher risk of Parkinson's disease across categories of lower LDL cholesterol levels (P=0.009 for categories and P=0.001 for fifths), but at most a borderline significant association between continuous LDL cholesterol levels and Parkinson's disease (hazard ratio for a 1 mmol/L lower LDL cholesterol level was 1.10 (95% confidence interval 1.00 to 1.21); P=0.05). This difference is probably because the risk of Parkinson's disease as a function of lower LDL cholesterol levels is not linear but only present at LDL cholesterol levels below 4.0 mmol/L.

Other studies
Current guidelines on treatment and prevention of cardiovascular disease recommend an LDL cholesterol level less than 1.8 mmol/L in patients at high cardiovascular risk, and less than 2.6 mmol/L in patients at high risk.[15] Very low LDL cholesterol levels could therefore be of concern, as cholesterol is a major constituent of the human brain and as low LDL cholesterol levels in theory could lead to dementia and Parkinson's disease. This concern is now even timelier, as novel PCSK9 inhibitors added to statins can reduce LDL cholesterol to very low levels.[5 6]

Reassuringly, however, extensive studies have described a normal neurological phenotype in a woman compound heterozygous for loss-of-function mutations in *PCSK9*, with no detectable expression of PCSK9 and with extremely low LDL cholesterol levels.[42] This observation is in accordance with the present findings of no consistent associations between low LDL cholesterol levels, observationally and genetically, and risk of Alzheimer's disease, vascular dementia, any dementia, or Parkinson's disease. In contrast with previous studies[10 13 43] we only found an increased observational risk of Parkinson's disease at low levels of LDL cholesterol and no increase in risk of dementia. An explanation for this could be that previous studies have been cross sectional, and severe neurological disease may change dietary intake and lifestyle, and thus LDL cholesterol levels. The increased observational risk of Parkinson's disease at low LDL cholesterol levels in our study may also be due to reverse causation, as preclinical Parkinson's disease may change lifestyle. It can be argued that it is unusual to conduct a Mendelian randomisation study when there is no observational association, as observed for dementia in our study. However, as statistically significant observational associations have been observed in previous studies,[10 13 43] it nevertheless seems relevant to conduct a Mendelian randomisation study. An observational low LDL cholesterol level is the net result of our genes,
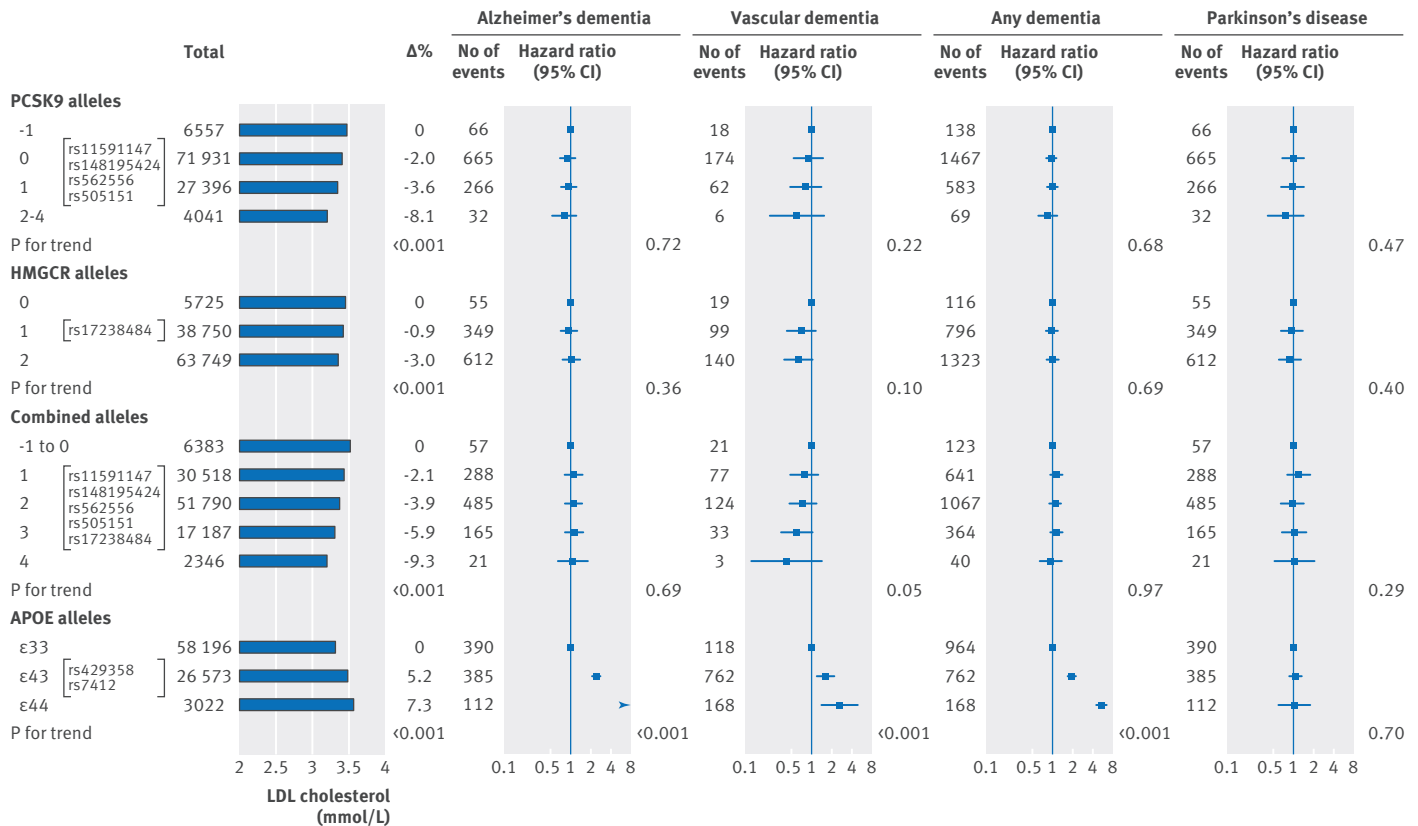
	Total	LDL cholesterol (mmol/L)	Δ%	Alzheimer's dementia		Vascular dementia		Any dementia		Parkinson's disease	
				No of events	Hazard ratio (95% CI)	No of events	Hazard ratio (95% CI)	No of events	Hazard ratio (95% CI)	No of events	Hazard ratio (95% CI)
PCSK9 alleles											
-1	6557		0	66		18		138		66	
0 (rs11591147, rs148195424, rs562556, rs505151)	71 931		-2.0	665		174		1467		665	
1	27 396		-3.6	266		62		583		266	
2-4	4041		-8.1	32		6		69		32	
P for trend			<0.001		0.72		0.22		0.68		0.47
HMGCR alleles											
0	5725		0	55		19		116		55	
1 (rs17238484)	38 750		-0.9	349		99		796		349	
2	63 749		-3.0	612		140		1323		612	
P for trend			<0.001		0.36		0.10		0.69		0.40
Combined alleles											
-1 to 0	6383		0	57		21		123		57	
1 (rs11591147, rs148195424, rs562556, rs505151, rs17238484)	30 518		-2.1	288		77		641		288	
2	51 790		-3.9	485		124		1067		485	
3	17 187		-5.9	165		33		364		165	
4	2346		-9.3	21		3		40		21	
P for trend			<0.001		0.69		0.05		0.97		0.29
APOE alleles											
ε33	58 196		0	390		118		964		390	
ε43 (rs429358, rs7412)	26 573		5.2	385		762		762		385	
ε44	3022		7.3	112		168		168		112	
P for trend			<0.001		<0.001		<0.001		<0.001		0.70

LDL cholesterol (mmol/L) axis: 2, 2.5, 3, 3.5, 4
Hazard ratio axes: 0.1, 0.5, 1, 2, 4, 8

Fig 3 | Prospective risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease as a function of *PCSK9, HMGCR*, and combined alleles adjusted for age, sex, and birth year. As a positive control of study power, risk of disease is also shown as a function of *APOE* ε43 and ε44 alleles compared with the common ε33 allele. Number of participants may vary owing to availability of genotypes. P values are from tests for trend of hazard ratios across ordered alleles. LDL=low density lipoprotein cholesterol; total=number of participants

dietary intake, use of drugs, and chronic diseases, whereas the genetically low LDL cholesterol levels examined using Mendelian randomisation represent lifelong low LDL cholesterol levels through a specific genetic pathway only—ie, via *PCSK9* or *HMGCR*.

Early on, PCSK9 was recognised as a potential drug target for lowering LDL cholesterol levels,[44 45] and efficacy and safety studies have shown that PCSK9 inhibition with monoclonal antibodies results in a 61-62% reduction in LDL cholesterol levels with few side effects.[5 6 14] In long term studies of PCSK9 inhibition, neurocognitive events have, however, been reported more frequently in people treated with evolocumab (0.9% v 0.3%) and alirocumab (1.2% v 0.5%) compared with placebo; although results have not been statistically significant.[5 6 14] The neurological symptoms reported have been diverse and unspecific, including cognitive and attention disorders and disturbances, amnesia, confusion, delirium, dementia, and disturbances in thinking and perception; some have occurred within 24 hours after onset of treatment; and the observed symptoms had no apparent relation to achieved LDL cholesterol concentrations.[5 6 14] Furthermore, a Mendelian randomisation study using the *PCSK9* R46L (rs11591147) variant, as also included in the present study, did not find an association with impaired cognitive performance or functional status in 5777 elderly participants in the

PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).[46]

For statins, and despite the fact that 2180 million prescriptions have been dispensed in the US since marketing began in 1987, effects on cognitive function remain controversial.[7-9 11] However, cognitive related adverse events have been reported to the US Food and Drug Administration at a rate of 1.9 per 1 million prescriptions, which is similar to rates seen with other commonly prescribed cardiovascular drugs.[47] Also, an exhaustive and systematic review using PubMed, Embase, Cochrane Library, and FDA databases concluded that in statin users there appeared to be: no increase in the incidence of Alzheimer's disease; no difference in cognitive performance related to procedural memory, attention, or motor speed; no increase in incidence of dementia or mild cognitive impairment; and no change in cognitive performance related to global cognitive performance scores, executive function, declarative memory, processing speed, or visuoperception.[47] However, another study also using FDA databases found that neurological disease was reported more often for lipophilic statins that more readily cross the blood-brain barrier, than for hydrophobic statins.[48] A meta-analysis of 23 randomised clinical intervention trials on statins reporting data on cognitive function in 29 012 participants failed to show adverse cognitive effects of statins and thus lower LDL
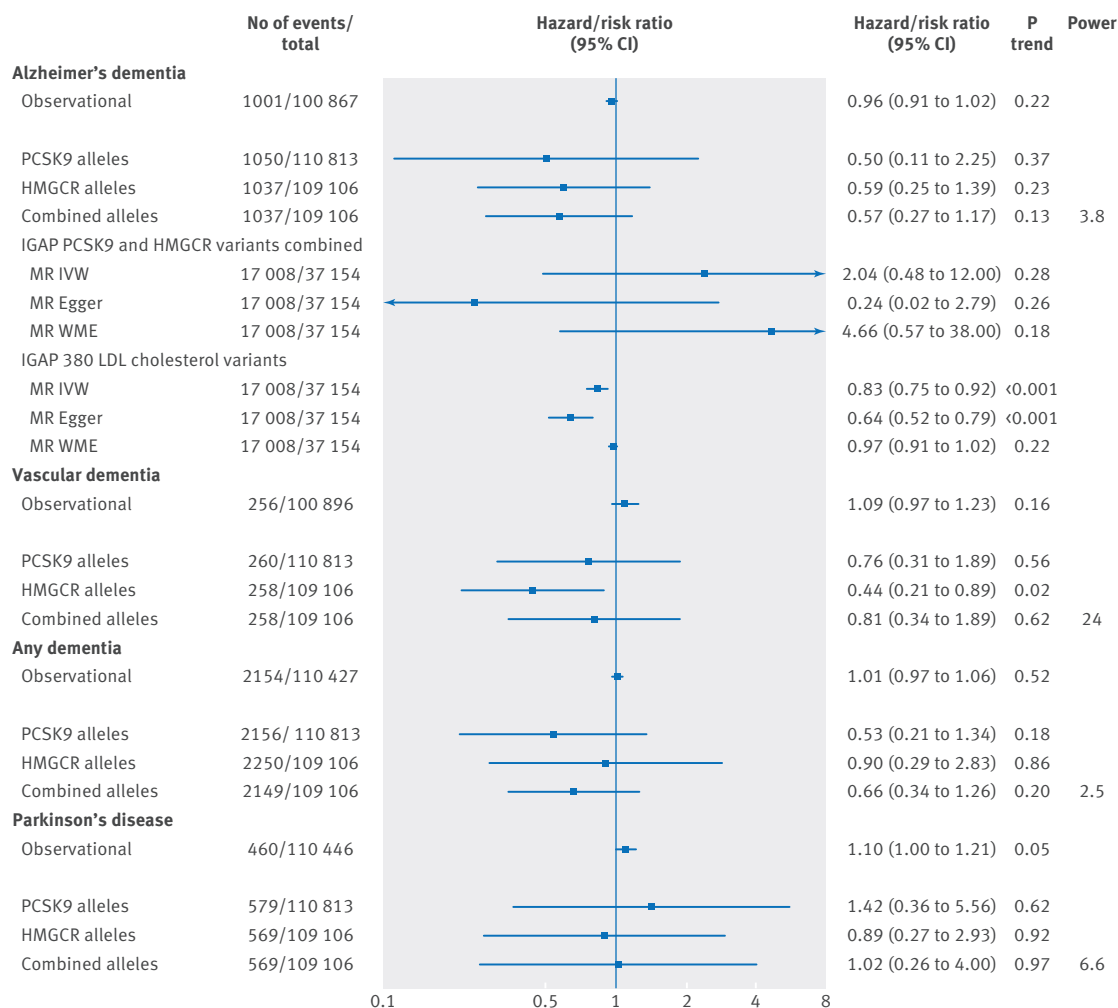
	No of events/ total	Hazard/risk ratio (95% CI)	Hazard/risk ratio (95% CI)	P trend	Power
Alzheimer's dementia					
Observational	1001/100 867		0.96 (0.91 to 1.02)	0.22	
PCSK9 alleles	1050/110 813		0.50 (0.11 to 2.25)	0.37	
HMGCR alleles	1037/109 106		0.59 (0.25 to 1.39)	0.23	
Combined alleles	1037/109 106		0.57 (0.27 to 1.17)	0.13	3.8
IGAP PCSK9 and HMGCR variants combined					
MR IVW	17 008/37 154		2.04 (0.48 to 12.00)	0.28	
MR Egger	17 008/37 154		0.24 (0.02 to 2.79)	0.26	
MR WME	17 008/37 154		4.66 (0.57 to 38.00)	0.18	
IGAP 380 LDL cholesterol variants					
MR IVW	17 008/37 154		0.83 (0.75 to 0.92)	‹0.001	
MR Egger	17 008/37 154		0.64 (0.52 to 0.79)	‹0.001	
MR WME	17 008/37 154		0.97 (0.91 to 1.02)	0.22	
Vascular dementia					
Observational	256/100 896		1.09 (0.97 to 1.23)	0.16	
PCSK9 alleles	260/110 813		0.76 (0.31 to 1.89)	0.56	
HMGCR alleles	258/109 106		0.44 (0.21 to 0.89)	0.02	
Combined alleles	258/109 106		0.81 (0.34 to 1.89)	0.62	24
Any dementia					
Observational	2154/110 427		1.01 (0.97 to 1.06)	0.52	
PCSK9 alleles	2156/ 110 813		0.53 (0.21 to 1.34)	0.18	
HMGCR alleles	2250/109 106		0.90 (0.29 to 2.83)	0.86	
Combined alleles	2149/109 106		0.66 (0.34 to 1.26)	0.20	2.5
Parkinson's disease					
Observational	460/110 446		1.10 (1.00 to 1.21)	0.05	
PCSK9 alleles	579/110 813		1.42 (0.36 to 5.56)	0.62	
HMGCR alleles	569/109 106		0.89 (0.27 to 2.93)	0.92	
Combined alleles	569/109 106		1.02 (0.26 to 4.00)	0.97	6.6

0.1 0.5 1 2 4 8

Fig 4 | Risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease for a 1 mmol/L lower observational and causal, genetically determined low density lipoprotein (LDL) cholesterol level. Hazard ratios (HR) for a 1 mmol/L lower observational LDL cholesterol levels were calculated using Cox regression and risk ratios (RR) for genetically lower LDL cholesterol levels were derived from instrumental variable analyses. For Alzheimer's disease, risk was also estimated using summary risk estimates from the International Genomics of Alzheimer's Project (IGAP) on genetic variants tested in the Global Lipid Genetics Consortium (GLGC) using either conventional inverse variance weighted Mendelian randomisation analysis (MR IVW), Egger Mendelian randomisation (MR Egger), or weighted median of instrumental variable estimates Mendelian randomisation (MR WME). Power denotes the odds ratio that can be excluded at a two sided α of 0.05 and β of 80% in a Mendelian randomisation design. P values are for significance of hazard and risk ratios. Total=number of participants

cholesterol levels during treatment.[13] This meta-analysis was specifically designed to meta-analyse cognitive test results and adverse event reports from randomised clinical intervention trials of statin treatment in cognitively healthy and impaired people in the short and long term. Taken together, there is no evidence for an association of low LDL cholesterol levels and risk of neurological disease from systematic reviews, meta-analysis of large randomised clinical intervention trials, and the present Mendelian randomisation study.

What the present study adds
Compared with previous observational data prone to confounding and reverse causation, and influenced by negative and positive stories on statins in the press,[49][50] our results using the Mendelian randomisation approach are completely free of reverse causations and largely free of confounding. Using such genetic data we could not detect any evidence that lifelong low LDL cholesterol levels had any harmful effects on risk of dementia and Parkinson's disease. If anything, low LDL cholesterol levels may lead to a low risk of Alzheimer's disease.

Implications of the present findings
With current recommendation for patients at high risk of cardiovascular disease to lower their LDL cholesterol levels below 1.8 mmol/L, and with conflicting reports on risk of neurological diseases in previous observational studies on lipid lowering, the finding of no causal harmful effect of low LDL cholesterol levels on risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease is important.

However, the present results should not be used to predict effects of specific lipid lowering drugs. Stringent cognitive studies are at present conducted in randomised clinical intervention trials of PCSK9 inhibitors,[56] and we await these findings before drawing firm conclusions.

Conclusion and future research

We used genetic variants in *PCSK9* and a variant in *HMGCR*, all lowering LDL cholesterol levels, to examine the causal effect of low LDL cholesterol levels on risk of neurological disease and did not observe any associations with increased risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease in 111 194 people from the general population. Data on 380 genetic variants from the International Genomics of Alzheimer's Project were, however, suggestive of a causal effect of low LDL cholesterol levels in reducing the risk of Alzheimer's disease. Beside randomised clinical intervention trials of PCSK9 inhibitors that are ongoing,[56] Mendelian randomisation studies such as the present one, but with more statistical power, are needed.

1   Baigent C, Blackwell L, Emberson J, et al. Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010;376:1670-81. doi:10.1016/S0140-6736(10)61350-5.

2   O'Regan C, Wu P, Arora P, Perri D, Mills EJ. Statin therapy in stroke prevention: a meta-analysis involving 121,000 patients. *Am J Med* 2008;121:24-33. doi:10.1016/j.amjmed.2007.06.033.

3   Mihaylova B, Emberson J, Blackwell L, et al. Cholesterol Treatment Trialists' (CTT) Collaborators. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet* 2012;380:581-90. doi:10.1016/S0140-6736(12)60367-5.

4   Kearney PM, Blackwell L, Collins R, et al. Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. *Lancet* 2008;371:117-25. doi:10.1016/S0140-6736(08)60104-X.

5   Sabatine MS, Giugliano RP, Wiviott SD, et al. Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015;372:1500-9. doi:10.1056/NEJMoa1500858.

6   Robinson JG, Farnier M, Krempf M, et al. ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015;372:1489-99. doi:10.1056/NEJMoa1501031.

7   Strom BL, Schinnar R, Karlawish J, Hennessy S, Teal V, Bilker WB. Statin Therapy and Risk of Acute Memory Impairment. *JAMA Intern Med* 2015;175:1399-405. doi:10.1001/jamainternmed.2015.2092.

8   Bernick C, Katz R, Smith NL, et al. Cardiovascular Health Study Collaborative Research Group. Statins and cognitive function in the elderly: the Cardiovascular Health Study. *Neurology* 2005;65:1388-94. doi:10.1212/01.wnl.0000182897.18229.ec.

9   Evans MA, Golomb BA. Statin-associated adverse cognitive effects: survey results from 171 patients. *Pharmacotherapy* 2009;29:800-11. doi:10.1592/phco.29.7.800.

10   Huang X, Alonso A, Guo X, et al. Statins, plasma cholesterol, and risk of Parkinson's disease: a prospective study. *Mov Disord* 2015;30:552-9. doi:10.1002/mds.26152.

11   Trompet S, van Vliet P, de Craen AJ, et al. Pravastatin and cognitive function in the elderly. Results of the PROSPER study. *J Neurol* 2010;257:85-90. doi:10.1007/s00415-009-5271-7.

12   Bai S, Song Y, Huang X, et al. Statin Use and the Risk of Parkinson's Disease: An Updated Meta-Analysis. *PLoS One* 2016;11:e0152564. doi:10.1371/journal.pone.0152564.

13   Ott BR, Daiello LA, Dahabreh IJ, et al. Do statins impair cognition? A systematic review and meta-analysis of randomized controlled trials. *J Gen Intern Med* 2015;30:348-58. doi:10.1007/s11606-014-3115-3.

14   Giugliano RP, Sabatine MS. Are PCSK9 Inhibitors the Next Breakthrough in the Cardiovascular Field? *J Am Coll Cardiol* 2015;65:2638-51. doi:10.1016/j.jacc.2015.05.001.

15   Piepoli MF, Hoes AW, Agewall S, et al. Authors/Task Force Members. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016;37:2315-81. doi:10.1093/eurheartj/ehw106.

16   Swerdlow DI, Preiss D, Kuchenbaecker KB, et al. DIAGRAM Consortium MAGIC Consortium InterAct Consortium. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *Lancet* 2015;385:351-61. doi:10.1016/S0140-6736(14)61183-1.

17   Stender S, Tybjærg-Hansen A. Using human genetics to predict the effects and side-effects of drugs. *Curr Opin Lipidol* 2016;27:105-11. doi:10.1097/MOL.0000000000000280.

18   Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008;27:1133-63. doi:10.1002/sim.3034.

19   Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;32:1-22. doi:10.1093/ije/dyg070.

20   Willer CJ, Schmidt EM, Sengupta S, et al. Global Lipids Genetics Consortium. Discovery and refinement of loci associated with lipid levels. *Nat Genet* 2013;45:1274-83. doi:10.1038/ng.2797.

21   Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. European Alzheimer's Disease Initiative (EADI) Genetic and Environmental Risk in Alzheimer's Disease Alzheimer's Disease Genetic Consortium Cohorts for Heart and Aging Research in Genomic Epidemiology. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 2013;45:1452-8. doi:10.1038/ng.2802.

22   Stender S, Nordestgaard BG, Tybjaerg-Hansen A. Elevated body mass index as a causal risk factor for symptomatic gallstone disease: a Mendelian randomization study. *Hepatology* 2013;58:2133-41. doi:10.1002/hep.26563.

23 Nordestgaard BG, Palmer TM, Benn M, et al. The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a Mendelian randomisation approach. *PLoS Med* 2012;9:e1001212. doi:10.1371/journal.pmed.1001212.

24 Varbo A, Benn M, Tybjærg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation* 2013;128:1298-309. doi:10.1161/CIRCULATIONAHA.113.003008.

25 Benn M, Tybjærg-Hansen A, Smith GD, Nordestgaard BG. High body mass index and cancer risk-a Mendelian randomisation study. *Eur J Epidemiol* 2016;31:879-92. doi:10.1007/s10654-016-0147-5.

26 Nordestgaard BG, Palmer TM, Benn M, et al. The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a Mendelian randomisation approach. *PLoS Med* 2012;9:e1001212. doi:10.1371/journal.pmed.1001212.

27 Frikke-Schmidt R, Nordestgaard BG, Thudium D, Moes Grønholdt ML, Tybjaerg-Hansen A. APOE genotype predicts AD and other dementia but not ischemic cerebrovascular disease. *Neurology* 2001;56:194-200. doi:10.1212/WNL.56.2.194.

28 Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of dementia in the general population. *Ann Neurol* 2015;77:301-11. doi:10.1002/ana.24326.

29 Phung TK, Andersen BB, Høgh P, Kessing LV, Mortensen PB, Waldemar G. Validity of dementia diagnoses in the Danish hospital registers. *Dement Geriatr Cogn Disord* 2007;24:220-8. doi:10.1159/000107084.

30 Jones PH, Davidson MH, Stein EA, et al. STELLAR Study Group. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). *Am J Cardiol* 2003;92:152-60. doi:10.1016/S0002-9149(03)00530-7.

31 Whitworth JA. World Health Organization, International Society of Hypertension Writing Group. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens* 2003;21:1983-92. doi:10.1097/00004872-200311000-00002.

32 Stata Statistical Software. *Release 13.*StataCorp LP, 2013.

33 Baum C, Schaffer M, Stillman S. Instrumental variables and GMM: Estimation and testing. *Stata J* 2003;3:1-31.

34 Nichols A. Ivpois: Stata module for IV/GMM Poisson regression. Stata/SE 13 1. 2007 http://ideas.repec.org/c/boc/bocode/s456890.html

35 Instrumental variables and GMM: estimation and testing. *Stata J* 2003;3:1-31.

36 Didelez V, Meng S, Sheehan NA. Assumptions of IV Methods for Observational Epidemiology. *Stat Sci* 2010;25:22-40doi:10.1214/09-STS316.

37 Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. *Int J Epidemiol* 2014;43:922-9. doi:10.1093/ije/dyu005.

38 Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013;37:658-65. doi:10.1002/gepi.21758.

39 Panagiotou OA, Ioannidis JP. Genome-Wide Significance Project. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. *Int J Epidemiol* 2012;41:273-86. doi:10.1093/ije/dyr178.

40 Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;44:512-25. doi:10.1093/ije/dyv080.

41 Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016;40:304-14. doi:10.1002/gepi.21965.

42 Zhao Z, Tuakli-Wosornu Y, Lagace TA, et al. Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. *Am J Hum Genet* 2006;79:514-23. doi:10.1086/507488.

43 Hu G, Antikainen R, Jousilahti P, Kivipelto M, Tuomilehto J. Total cholesterol and the risk of Parkinson disease. *Neurology* 2008;70:1972-9. doi:10.1212/01.wnl.0000312511.62699.a8.

44 Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003;34:154-6. doi:10.1038/ng1161.

45 Cohen JC, Boerwinkle E, Mosley TH Jr,, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006;354:1264-72. doi:10.1056/NEJMoa054013.

46 Postmus I, Trompet S, de Craen AJ, et al. PCSK9 SNP rs11591147 is associated with low cholesterol levels but not with cognitive performance or noncardiovascular clinical events in an elderly population. *J Lipid Res* 2013;54:561-6. doi:10.1194/jlr.M033969.

47 Richardson K, Schoen M, French B, et al. Statins and cognitive function: a systematic review. *Ann Intern Med* 2013;159:688-97. doi:10.7326/0003-4819-159-10-201311190-00007.

48 Sahebzamani FM, Munro CL, Marroquin OC, Diamond DM, Keller E, Kip KE. Examination of the FDA Warning for Statins and Cognitive Dysfunction. *J Pharmacovigil* 2014;2:e1000141 doi:10.4172/2329-6887.1000141.

49 Nielsen SF, Nordestgaard BG. Negative statin-related news stories decrease statin persistence and increase myocardial infarction and cardiovascular mortality: a nationwide prospective cohort study. *Eur Heart J* 2016;37:908-16. doi:10.1093/eurheartj/ehv641.

50 Matthews A, Herrett E, Gasparrini A, et al. Impact of statin related media coverage on use of statins: interrupted time series analysis with UK primary care data. *BMJ* 2016;353:i3283. doi:10.1136/bmj.i3283.

Supplementary figure: Mendelian randomisation study design

Supplementary information: additional tables and figures