Clinical research

Single nucleotide polymorphism in the low-density lipoprotein receptor is associated with a threefold risk of stroke

A case-control and prospective study

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Background

More than 600 different, but rare, mutations in the low-density lipoprotein (LDL) receptor have been identified as the cause of familial hypercholesterolaemia. In contrast, only a single common amino acid-changing polymorphism (A370T) has been reported in this gene. The association of this polymorphism with variations in lipid levels is at present unclear.

Methods

We obtained genotypes for 9238 individuals from The Copenhagen City Heart Study, of which 465 had stroke and 1019 had ischaemic heart disease.

Results

In this cohort from the Danish general population, 90.2% (n = 832), 9.5% (n = 875), and 0.3% (n = 31) were 370A homozygotes, A370T heterozygotes, and 370T homozygotes, respectively. The incidences of stroke in 370A homozygotes, A370T heterozygotes, and 370T homozygotes were 28, 26, and 100 per 10,000 person–years, respectively (370T homozygotes vs. 370A homozygotes: log-rank, P = 0.002). The relative risk and odds ratio for stroke in 370T homozygotes vs. 370A homozygotes were 3.6 (95% confidence interval, 1.5–8.8) and 3.6 (95% confidence interval, 1.3–9.8) in prospective and cross-sectional studies, respectively. Furthermore, average age at onset of stroke in 370T homozygotes tended to be lower than in heterozygotes and 370A homozygotes combined (59 vs. 66 years, P = 0.08). In contrast, neither levels of cholesterol, LDL cholesterol, apolipoprotein B, or triglycerides, nor risk of ischaemic heart disease was associated with genotype.

Conclusion

This is the first prospective study to suggest an association between a polymorphism in the LDL receptor and stroke. Because this association is independent of lipid levels, our results point toward a hitherto unknown function of this receptor in the brain.

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Introduction

The low-density lipoprotein (LDL) receptor family comprises several receptors with variable expression and function in tissues. The most studied so far is the LDL receptor (LDLR) that is responsible for the uptake and degradation of apolipoprotein E (apoE) and apolipoprotein B (apoB) containing lipoproteins.1

Mutations in the LDLR gene may lead to dysfunction of the receptor and familial hypercholesterolaemia, and eventually to premature ischaemic heart disease. More than 600 different, but rare, mutations in the LDLR have been associated with familial hypercholesterolaemia. By contrast, only one amino acid-changing single nucleotide polymorphism (SNP) in the LDLR has been reported (http://www.ucl.ac.uk/fh/polypage.html). This SNP, A370T, which is caused by a G-to-A substitution at position 1184 in exon 8, was first reported in a South African white population,2 and has been associated previously with increased cholesterol levels in one small study.3

In brain tissue, members of the LDLR family are responsible for the cellular uptake of apoE, β-amyloid precursor protein, matrix proteins, hepatic- and lipoprotein lipases, and several proteinase/proteinase inhibitor complexes (e.g., clotting factors/inhibitors, α-2-macroglobulin, and α-1-antichymotrypsin).4,8 LDLR itself is expressed in the brain on capillary endothelial cells,9 on astrocytes in white matter and on brainstem cells, and to a lesser extent in the cells of the cortical grey matter.10,11 It is therefore conceivable that mutations in the LDLR gene could influence the risk of cerebrovascular events through pathways other than atherosclerosis, although this has not yet been shown.

To ascertain the possible association between the common A370T polymorphism in the LDLR gene and variations in lipid and lipoprotein levels, as well as risk of stroke and ischaemic heart disease, we obtained genotypes of 9238 individuals from a general population sample, The Copenhagen City Heart Study, of which 465 had stroke and 1019 had ischaemic heart disease.

Methods

Subjects

The Copenhagen City Heart Study is a prospective cardiovascular population study of 20–80-year-old women and men randomly selected to reflect the general adult Danish population. In 1976–1978, 19,329 individuals were invited to participate, of whom 74% (14,223) accepted. In 1981–1983, the original cohort, supplemented with 500 subjects 20–25-years-old, were invited to participate and 70% (12,698) accepted. Finally, in 1991–1994, the cohort, further supplemented with 3000 subjects 20–49-years-old, were invited to participate and 61% (10,135) accepted.12,13 At the 1991–1994 examination, additional blood samples for DNA extraction were drawn. DNA was available for 9259 participants and the genotype for the A370T SNP in the LDL receptor was obtained in 9238 of them.

Four hundred and sixty-five individuals (220 women and 245 men) with previous sudden onset of focal neurologic symptoms due to cerebrovascular disease were identified by an experienced neurologist who was unaware of the results of genotyping, on the basis of history and review of all hospital admissions and diagnoses entered in the Danish National Hospital Discharge Register, and of medical records from hospitals or general practitioners. The diagnoses were based on the International Classification of Diseases, 8th and 10th revisions (cerebrovascular disease codes 431 through 438, and I61 through I69). Cerebrovascular events were defined as cerebral infarction (n = 349), haemorrhagic stroke (n = 29), transient ischaemic attack (focal neurologic symptoms lasting less than 24 hours; n = 79), or amaurosis fugax (transient blindness in only one eye; n = 8), a total of 465 cases. For the sake of simplicity, henceforth we will refer to these cerebrovascular events as "stroke". Subgroup analyses were made of cases with cerebral infarction or transient ischaemic attack without atrial fibrillation (393 and 369 cases for cross-sectional and prospective analyses, respectively) to exclude the most obvious thromboembolic strokes. Participants were screened for manifestations of ischaemic heart disease by reviewing all hospital admissions and diagnoses entered in the Danish National Hospital Discharge Register. The diagnoses were based on the International Classification of Diseases, 8th and 10th revisions (ischaemic heart disease codes 410 through 414, and I20.0 through I25.9, respectively). One thousand and nineteen individuals (413 women and 606 men) had ischaemic heart disease according to the above criteria. Diabetes and hypertension were diagnosed as described previously.14 The association of genotype with variations in lipid and lipoprotein levels was determined in the general population sample (n = 9238) after excluding individuals on lipid-lowering medication (n = 187).

More than 99% of subjects were white and nearly 99% were of Danish ancestry. All participants gave informed consent and the study was approved by the relevant ethics committees.

Study designs

Cross-sectional studies

The cohort was defined as participants in The Copenhagen City Heart Study who were seen in the 1991–94 examination and underwent genotyping for A370T in the LDLR (n = 9238). Cases included participants with stroke verified up until 1998 (n = 465) or ischaemic heart disease verified up until 1999 (n = 1019). Controls included all participants without stroke (n = 8432) or without ischaemic heart disease (n = 7559), but within the same age range as cases: 28–90 years for stroke (excluding 341 participants) and 33–90 years for ischaemic heart disease (excluding 660 participants).

Prospective studies

The cohort was defined as above, except that outcomes (stroke and ischaemic heart disease) were recorded in the follow-up period from 1976 through 1999, and individuals diagnosed with either endpoint before entry were excluded, leaving 438 incident stroke cases and 956 incident ischaemic heart disease cases for the prospective analyses. Thus, compared with the cross-sectional study, 27 stroke cases and 63 cases of ischaemic heart disease had events before study entry and were excluded from the prospective analyses.

DNA analyses

The A370T SNP consists of the substitution of guanine for adenine (G → A) at position 1184 of complementary DNA in exon 8 of the LDLR gene.15 A 150-bp DNA fragment spanning the site of the mutation was amplified by polymerase chain reaction (PCR) using two flanking primers (P1: 5'-GAGTGTCAAGGATCCCGAG-3'; P2: 5'-GAGTGTCAAGGATCCCGAG-3').
CACCTGGCCG-3′, 1 μmol/litre; P2: 5′-AAGTCGACCCACCCGGGC- 
CCCTTCCGT-3′, 0.75 μmol/litre); the insertion of a mismatch in 
P1 creates an internal control site for HaeIII. The annealing 
temperature was 68 °C and reactions were performed in a total 
volume of 30 μl with the primer concentrations indicated: 0.6 U 
of Taq polymerase, 200 μmol of each deoxynucleotidetriphos- 
phate per litre, 0.75 mmol of magnesium chloride per litre, 1 × 
buffer (200 mM Tris–hydrochloride, pH 8.4, and 500 mM po-
tassium chloride), and 0.1–0.2 μg DNA. PCR products were di-
gested with HaeIII; the presence of the SNP destroys a HaeIII 
site. A common band of 26 bp and bands of either 47 and 77 bp 
(SNP absent) or 124 bp (SNP present) were separated on 3.5% 
agarose gel and stained with ethidium bromide. All homozygotes 
were restested in at least one separate restriction analysis and no 
discrepancies were identified.

ApoE genotypes were identified by PCR followed by digestion 
with HhaI and HaeIII in all 9238 individuals, as previously de-
scribed.16 17

Other analyses

Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, high-density lipoprotein 
(HDL) cholesterol, triglycerides, and apoB (all Boehringer 
Mannheim, Mannheim, Germany); LDL cholesterol levels were 
calculated using the equation of Friedewald et al.18

Statistical analysis

Data were analysed using the SPSS program. A P value <0.05 
on a two-sided test was considered significant. P values were not 
corrected for multiple comparisons. Continuous variables be-
tween patient groups and controls were compared using the 
Mann–Whitney U test. Differences in categorical values were 
tested by Pearson’s χ² test. Analysis of covariance (ANCOVA) 
was used to adjust levels of cholesterol, LDL cholesterol, apoB, 
and triglycerides for age in 10-year age groups. Residuals for 
these models were saved and subtracted from the original un-
adjusted values, and then saved in a new age-adjusted choles-
terol, LDL cholesterol, apoB, and triglyceride variable. The 
association between genotype and the adjusted values was de-
termined in a Kruskal–Wallis analysis of variance (ANOVA). To 
determine whether the association of the LDLR genotype with 
levels of cholesterol, LDL cholesterol, apoB, and triglycerides 
were dependent on apoE genotype, we stratified lipids and lipo-
proteins by the three most common apoE genotypes (e2, e32 and 
e43) and applied Kruskal–Wallis ANOVA across the A370T 
genotypes.

Cross-sectional data

Logistic regression analysis20 21 exploring the association of 
A370T polymorphism with disease (stroke or ischaemic heart 
disease) was performed (patients with stroke or ischaemic heart 
disease in the general population sample vs. controls without 
stroke or ischaemic heart disease in the general population 
sample). Cases were compared with controls within the same 
age range and age in 10-year age groups was added to the 
model. Furthermore, multifactorial logistic regression models 
including age, total cholesterol, LDL cholesterol, apoB, and 
triglycerides examined if associations of the A370T polymor-
phism with stroke or ischaemic heart disease in the general 
population were dependent on lipids and lipoproteins. Finally, 
these models were repeated with the addition of diabetes mellitus, 
hypertension, and smoking status. The homogeneity of the 
association of genotype and conventional cardiovascular risk 
factors (age, gender, lipids and lipoproteins, hypertension, 
diabetes mellitus, smoking, and apoE genotype) in the predic-
tion of stroke or ischaemic heart disease in the general popu-
lation was tested by introducing all possible two-factor inter-
action terms between genotype and the above-mentioned 
facets one at a time. Overall model fit for covariates or inter-
action terms was tested using the likelihood ratio test between 
the complete and reduced models. The contribution of LDLR 
A370T genotype in predicting the dependent variable (stroke 
or ischaemic heart disease) when the independent variables 
were allowed for was expressed as an odds ratio (e²) with 95% 
confidence intervals (e²±1.96×SEM)21, where β is the regression 
coefficient.

Prospective data

The log-rank test was performed to test whether the cumulative 
incidence of stroke or ischaemic heart disease was dependent on 
genotype, and these data were plotted as Kaplan–Meier curves. 
The relative risk for disease with 95% confidence intervals was 
calculated using Cox regression adjusted for age at entry into The 
Copenhagen City Heart Study. The proportional hazards as-
sumption was tested by inspection of log-minus-log curves, as-
suring that hazards did not change over time for any covariate. 
Individuals with events before entry were excluded (27 stroke 
cases and 63 cases with ischaemic heart disease when compared 
with the cross-sectional study).

Role of the funding source

The funding source had no role in planning the study design, in 
the collection, analysis and interpretation of data, in writing the 
report, or in the decision to submit the paper for publication.

Results

Characteristics of patients with stroke or ischaemic heart 
disease and of controls from The Copenhagen City Heart 
Study are shown in Table 1 for each gender separately. Female 
patients were older and had significantly higher cholesterol, 
LDL cholesterol, and triglyceride levels than controls. Male patients with ischaemic heart disease also had significantly lower HDL cholesterol levels and 
were more obese than controls. Male patients were older 
and had significantly lower levels of HDL cholesterol and 
significantly higher levels of triglycerides. In addition, 
male patients with ischaemic heart disease had higher cholesterol and LDL cholesterol levels and were more 
obese than controls. Both female and male patients more often 
had hypertension and diabetes mellitus than controls.

In the general population sample as a whole (n = 9238), the relative frequency of A370T heterozygo-

tes was 0.095 (n = 875) and the relative frequency of 
370T homozygotes was 0.003 (n = 31). Genotype fre-
cuencies did not differ significantly from those predicted 
by the Hardy–Weinberg equilibrium (v² : P = 0.12) for 
the population as a whole. Genotype frequencies in the stroke 
and ischaemic heart disease groups differed significantly 
from those predicted by the Hardy–Weinberg equilibrium 
(v² : stroke group, P = 0.004; ischaemic heart disease 
group, P = 0.03). For this reason, all 370T homozygotes 
were evaluated for possible laboratory error by renewed 
restriction analysis, but no discrepancies were identified.
Association of genotype with lipid and lipoprotein levels in the general population

Age-adjusted levels of total cholesterol, LDL cholesterol, apoB, and triglycerides did not differ significantly as a function of genotype in the general population as a whole \((P = 0.86; P = 0.40; P = 0.62; P = 0.63\) \(P = 0.01\). \(P = 0.01\) (Fig. 1 and data not shown), or upon stratifying by gender (data not shown). When stratification was performed by the three most common apoe genotypes \((i32, i33, i43)\), there was no association of A370T genotype with variations in lipid or lipoprotein levels for cholesterol \((i32, P = 0.19; i33, P = 0.95; i43, P = 0.35)\), LDL cholesterol \((i32, P = 0.10; i33, P = 0.36; i43, P = 0.21)\), apoB \((i32, P = 0.09; i33, P = 0.81; i43, P = 0.07)\), or triglycerides \((i32, P = 0.71; i33, P = 0.45; i43, P = 0.49)\).

Association of genotype with risk of stroke and ischaemic heart disease in the general population

Cross-sectional data

On univariate analysis, the relative frequency of 370T homozygotes was significantly higher in patients with stroke than in controls \((\chi^2 : P = 0.02)\), whereas the relative frequency in patients with ischaemic heart disease did not differ significantly from controls \((\chi^2 : P = 0.3)\) (data not shown). On multifactorial logistic regression analysis allowing for age, the risk of stroke was more than threefold greater in individuals homozygous for 370T compared with homozygous carriers of 370A (odds ratio: 3.6, 95% confidence interval 1.3–9.8; \(P = 0.01\)) (Fig. 2, upper panel), and remained significant after adjusting for multiple comparisons (significance level after Bonferroni correction: 0.05/3 genotype comparisons = 0.015). This association did not change when cholesterol, LDL cholesterol, apoB, triglycerides, diabetes, hypertension, or smoking were added to the logistic regression model. In contrast, risk of ischaemic heart disease was not associated with A370T genotype. Results were similar when apoe genotype was taken into account in the regression models (data not shown). Genotype did not interact with age, gender, apoE genotype, diabetes, hypertension, or smoking in predicting stroke or ischaemic heart disease in the general population.

Prospective data

We detected 438 incident strokes and 956 incident ischaemic heart disease events during 22 years of follow-up. The incidences of stroke and ischaemic heart disease in AA homozygotes, AT heterozygotes, and TT homozygotes were 28, 26, and 100 and 59, 57, and 107 per 10,000 person–years, respectively. In accordance with this, the cumulative incidence of stroke was higher in TT homozygotes than in AA homozygotes (Fig. 2, lower

Table 1 Characteristics of patients with stroke and/or ischaemic heart disease and of controls in the same age range from The Copenhagen City Heart Study

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 465)</th>
<th>Ischaemic heart disease (n = 1019)</th>
<th>Controls (n = 8432)</th>
<th>No stroke (n = 7559)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.2 ± 0.6</td>
<td>69.8 ± 0.4</td>
<td>75.6 ± 0.2</td>
<td>75.9 ± 0.2</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>6.9 ± 0.08</td>
<td>6.8 ± 0.07</td>
<td>8.3 ± 0.02</td>
<td>8.4 ± 0.02</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>4.2 ± 0.08</td>
<td>4.20 ± 0.06</td>
<td>3.8 ± 0.02</td>
<td>3.8 ± 0.02</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.7 ± 0.04</td>
<td>1.6 ± 0.03</td>
<td>1.7 ± 0.01</td>
<td>1.7 ± 0.01</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.1 ± 0.08</td>
<td>2.1 ± 0.06</td>
<td>1.7 ± 0.02</td>
<td>1.7 ± 0.02</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.8 ± 0.3</td>
<td>26.3 ± 0.3</td>
<td>25.3 ± 0.07</td>
<td>25.3 ± 0.07</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>41</td>
<td>39</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>47</td>
<td>47</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Males (n)</td>
<td>245</td>
<td>606</td>
<td>3,725</td>
<td>3,202</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.3 ± 0.6</td>
<td>67.6 ± 0.40</td>
<td>57.2 ± 0.2</td>
<td>57.5 ± 0.2</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>6.1 ± 0.07</td>
<td>6.3 ± 0.05</td>
<td>6.0 ± 0.02</td>
<td>6.0 ± 0.02</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.8 ± 0.07</td>
<td>3.9 ± 0.04</td>
<td>3.6 ± 0.02</td>
<td>3.6 ± 0.02</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.03</td>
<td>1.3 ± 0.02</td>
<td>1.4 ± 0.01</td>
<td>1.4 ± 0.01</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.3 ± 0.08</td>
<td>2.3 ± 0.07</td>
<td>2.1 ± 0.03</td>
<td>2.1 ± 0.04</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.5 ± 0.2</td>
<td>26.8 ± 0.2</td>
<td>26.2 ± 0.06</td>
<td>26.2 ± 0.07</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>41</td>
<td>32</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>54</td>
<td>52</td>
<td>53</td>
<td>54</td>
</tr>
</tbody>
</table>

Values in the upper part of the table are means ± SEM. Mann–Whitney U tests and \(\chi^2\)-tests were used to compare continuous and categorical variables in patients with stroke and ischaemic heart disease with controls in The Copenhagen City Heart Study without stroke or ischaemic heart disease.

Controls were within the same age ranges as patients: stroke: 28–90 years (excluding 341 participants), ischaemic heart disease: 33–90 years (excluding 660 participants).

\(P < 0.05\).

\(P < 0.001\).
Subgroup analyses
In an attempt to differentiate between ischaemic and thromboembolic stroke, subgroup analyses were performed, including only stroke cases with ischaemic stroke or transient ischaemic attack without atrial fibrillation (n = 393 and n = 369 cases for the cross-sectional and prospective studies, respectively), and thus excluding the most obvious thromboembolic stroke categories. The association between homozygosity for the 370T allele and risk of stroke remained significant, and improved in both the cross-sectional and prospective studies (OR 4.1, 95% confidence interval 1.5–11.2; RR 4.2, 95% confidence interval 1.7–10.1).

Characteristics of individuals homozygous for 370T
Characteristics of the 31 individuals homozygous for 370T are shown in Table 2. There were no significant differences in age, levels of cholesterol, LDL cholesterol, and apoB, or in the frequencies of hypertension, diabetes mellitus, smoking, or ischaemic heart disease between the five homozygous individuals with stroke and the 26 homozygous individuals without stroke. The frequency of stroke in the 31 TT homozygotes was 16.1% compared with 5.5% in heterozygotes and AA homozygotes in the general population (P = 0.009). The average age at onset of stroke was 59 years in 370T homozygotes compared with 66 years in the general population (P = 0.08), pointing to a possible association of this genetic variant with stroke. Three of the five patients with stroke also had ischaemic heart disease with age at onset 5–14 years after their stroke; 2 of these patients had severe hypercholesterolaemia (patients numbers 2 and 4).

Discussion
Among Danes, the majority of patients with clinical familial hypercholesterolaemia are carriers of different mutations in the LDLR gene, all of them rare.22 By contrast, only a single amino acid-changing SNP (A370T) has been reported in the LDLR gene (http://www.ucl.ac.uk/fh/polypage.html). Although not associated with familial hypercholesterolaemia, it is unclear whether this SNP might have a more moderate effect on cholesterol levels in the general population.3

Association of genotype with lipids, lipoproteins, and risk of ischaemic heart disease in the general population
In the present study, A370T SNP was not associated with variations in lipids or lipoproteins, or with risk of ischaemic heart disease. The A370T substitution is caused by a G-to-A substitution in exon 8 of the LDLR gene, in the epidermal growth factor (EGF) precursor homology
region, repeat B. This region of multiple copies of a 40-amino acid, cysteine-rich sequence is involved in acid-dependent dissociation of ligand and receptor in the endosomes prior to recycling of the receptor to the cell surface. The majority of rare mutations associated with familial hypercholesterolaemia in this part of the gene result biochemically in type V defects, that is, the production of receptors that bind and internalise LDL, but fail to recycle to the cell surface (recycling-defective alleles).\(^1\) Similar growth factor repeats have now been identified in other members of the LDLR family, in proteins of the blood clotting system, in growth factors, and in proteins involved in Notch signalling.\(^2\)\(^-\)\(^5\) It is suggested that EGF repeats help to mediate acid-dependent conformational changes or protein-protein interactions,\(^2\)\(^-\)\(^4\) for example, homo- or heterodimerisation.\(^2\)\(^6\) The lack of an association between 370T and cholesterol levels in the present study suggests that this site is not, as discussed above, pivotal for acid-dependent dissociation, but rather might affect protein–protein interaction.

Gudnason et al.\(^3\), in contrast, found an association between A370T heterozygosity and hypercholesterolaemia in men in a small Icelandic population. However, in women in the same study almost the opposite effect appeared, and there were no homozygotes included in the study. In support of our findings, these authors did not find any difference in uptake and degradation of LDL.

![Cross-sectional data](https://academic.oup.com/eurheartj/article-abstract/25/11/943/422974/fig2)

**Fig. 2** Upper panel, cross-sectional data. Risk of stroke and of ischaemic heart disease in the general population as a function of A370T genotype. Risk is presented as an odds ratio with 95 percent confidence intervals from logistic regression analyses and relative to AA homozygotes. Lower panel, prospective data. Cumulative incidence of stroke and ischaemic heart disease in the general population as a function of 22 years of follow-up according to A370T genotype. The log-rank test was performed to determine whether the cumulative incidence of stroke or ischaemic heart disease was dependent on A370T genotype. The relative risk for disease with 95% confidence interval was calculated using Cox regression adjusted for age at entry into the Copenhagen City Heart Study. AA = homozygous for 370A (wild type), AT = heterozygous, TT = homozygous for 370T.
cholsterol between mutant and wild-type receptors in vitro, suggesting that the A370T SNP was not the cause of hypercholesterolaemia in these individuals.

Association of genotype with stroke in the general population

Very little is known about the genetics of stroke, although in some cases there is clearly a genetic component. Rare mutations in the Notch 3 gene are to some degree responsible for the CADASIL syndrome, cerebral autosomal-dominant arteriopathy with subcortical infarcts, and leukoencephalopathy. So far, only common SNPs in fibrinogen, lipoprotein lipase, and apoE have consistently been associated with stroke or dementia.

The members of the LDLR family are LDLR, LDLR-receptor (VLDLR), apolipoprotein E receptor2 (APOER2), and megalin. A common characteristic of all family members is the ability to bind apoE. LRP and megalin are multifunctional receptors that bind a multitude of structurally different proteins and, in addition, seem to have important functions in the brain, either in the maintenance of, or embryonic development of brain tissue. Mutations in either LRP or VLDLR genes or ligands of these receptors, the most important being apoE, have been associated with Alzheimer’s disease independent of lipid levels. Although the multifunctional receptors of the LDLR family and APOER2 seem to be more important in the brain than LDLR, LDLR is known to be expressed on endothelial cells in brain capillaries, and on astrocytes in white matter, brainstem cells, and to a lesser extent on the cells of the cortical grey matter. It is known that at least LRP is responsible for the catabolism of various clotting factors and inhibitors, factor IXa, Xla, tissue factor pathway inhibitor (TFPI), free tissue plasminogen activator (tPA), and tPA-plasminogen activator inhibitor 1 (PAI-1) complexes, but at present it is not known whether LDLR has similar functions.

The lack of a genotype effect on cholesterol levels does not preclude an effect on risk of stroke. Cholesterol is not an independent risk factor for stroke in The Copenhagen City Heart Study as in many other studies (although it is a risk factor for ischaemic heart disease), probably because the majority of strokes are not atherosclerotic. Furthermore, stroke is not overrepresented in families with familial hypercholesterolaemia due to rare LDL-receptor mutations. As is the case for the apoE e4 allele association with Alzheimer’s disease and the present prospective findings, a clear intermediate phenotype for CADASIL patients with Notch 3 mutations has not been established. Mutations in CADASIL patients always involve either loss or gain of a cysteine residue in the EGF repeat. A370T in the LDLR gene introduces a threonine just before the last of six highly conserved cysteine residues thought to form three internal disulfide bonds, and might lead to disrupted disulfide bonds and, thus, conformational changes. The importance of the 370 position of the LDLR is further emphasised by the high degree of phylogenetic conservation.

In light of this, it is conceivable that the threefold greater risk of stroke found in 370T homozygotes is not associated with increased cholesterol levels, but might potentially alter protein–protein interactions, such as interactions with clotting factors or the Notch 3 protein, perhaps leading to a prothrombotic state in brain capillaries or a hyperproliferative state of vascular smooth muscle cells in cerebral arteries. In the present study, exclusion of potential thromboembolic events appeared to strengthen the findings, and thus might support speculations on a prothrombotic state locally in brain capillaries. However, as is the case for the relation between the apoE e4 allele and Alzheimer’s disease and the Notch 3 gene and the CADASIL stroke syndrome, the exact mechanism is at present unknown.

Table 2 Characteristics of 31 individuals homozygous for the A370T mutation in exon 8 of the low-density lipoprotein receptor gene

<table>
<thead>
<tr>
<th>Stroke</th>
<th>No stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>M</td>
</tr>
<tr>
<td>ApoE genotype</td>
<td>i3/i3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>6.0</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>4.4</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>100</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2.3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes</td>
</tr>
<tr>
<td>Diabetes</td>
<td>No</td>
</tr>
<tr>
<td>Smoking</td>
<td>No</td>
</tr>
<tr>
<td>Stroke</td>
<td>Stroke</td>
</tr>
<tr>
<td>Age at onset</td>
<td>61</td>
</tr>
<tr>
<td>Ischaemic heart</td>
<td>–</td>
</tr>
<tr>
<td>Age at onset</td>
<td>–</td>
</tr>
</tbody>
</table>

Values for homozygous individuals without stroke are means ± SEM. AMI, acute myocardial infarction; F, female; IHD, ischaemic heart disease; M, male; TIA, transient ischaemic attack. Mann–Whitney U tests and χ² tests were used to compare continuous and categorical variables in patients with stroke with persons without stroke.
Strengths and limitations of study

A major strength of our study is the prospective population-based design comprising a large cohort of the general population with a follow-up of approximately 22 years and an exposure time for stroke of 157,263 person-years and for ischaemic heart disease of 161,893 person-years. To our knowledge, this is by far the largest study to date examining the association of a common SNP in the LDLR gene with lipid levels and risk of stroke and ischaemic heart disease. It is therefore also the only study including a large number of 370T homozygotes, which may explain why an association with stroke has not been identified previously.

An important limitation of the present study is the fact that we can only speculate on the precise function of the mutated A370T LDLR protein. Crystallographic models of the LDLR are only available for the ligand-binding domain (exons 2–6). For this reason, it has not been possible to generate computerised mutagenesis involving this SNP.

A second important limitation is the fact that genotyping was performed only in the participants that attended the third examination of The Copenhagen City Heart Study from 1991 to 1994. Thus, selection bias may have occurred if death or severe disease prevented these persons from undergoing genotyping. The patients who completed follow-up in 1991 to 1994 had an incidence of stroke of 33 per 10,000 person-years and an incidence of ischaemic heart disease of 70 per 10,000 person-years. The patients who did not complete follow-up in 1991–1994, and thus were not included in this study, had corresponding incidence rates of stroke of 96 per 10,000 person-years and of ischaemic heart disease of 202 per 10,000 person-years. However, the approximate threefold increase in incidence rates for both stroke and ischaemic heart disease for participants who did not complete follow-up in 1991–1994 compared to those who did at least partly reflects the fact that the mean age of non-attenders was, on average, 8 years higher than the mean age of attenders (56 vs. 48 years). Furthermore, this increase in incidence was not dependent on genotype, since genotype frequencies in the attenders did not change as a function of age, and genotypes in the overall sample were in Hardy–Weinberg equilibrium. Thus, we do not consider selection bias against TT homozygosity likely. Nevertheless, if this had occurred in our study, it would be more likely to result in a conservative estimate of the association of the homozygous 370T genotype with stroke, and would underestimate rather than overestimate this association.

Conclusion

This is the first study to suggest that a common SNP in the LDLR gene might be associated with an increased risk of stroke, independent of lipid levels, and perhaps with an earlier onset of disease in a white general population. Our results may point toward a hitherto unknown function of this receptor in the brain.