Heterozygosity for R1141X in ABCC6 and Risk of Ischemic Vascular Disease

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Background—Pseudoxanthoma elasticum (PXE) is an autosomal recessive disease caused by loss-of-function mutations in ABCC6 and characterized by elastic calcification leading to dermal, ocular, and ischemic vascular disease. We tested the hypothesis that heterozygosity for R1141X, the most frequent PXE-causing mutation in Caucasians, associated with risk of ischemic vascular disease, as previous studies suggested 4- to 11-fold risk of ischemic heart disease (IHD) in heterozygotes.

Methods and Results—We studied 10,276 persons from the general population, including 1985 with IHD and 989 with ischemic cerebrovascular disease (ICVD). We examined 45,603 individuals from a cross-sectional general population study, of whom 3738 had IHD and 2335 had ICVD. Finally, we compared 4851 patients with IHD and 625 patients with ICVD with, respectively, 4851 and 625 matched control subjects. We genotyped participants in all studies for ABCC6 R1141X. The frequency of R1141X was 0.6% in all populations studied. ABCC6 R1141X genotype was not associated with an increased risk of IHD, myocardial infarction, ICVD, or ischemic stroke. Furthermore, R1141X genotype did not interact with age on risk of the largest end point, IHD. Finally, R1141X genotype did not associate with variation in plasma levels of high-sensitivity C-reactive protein, fibrinogen, blood pressure, or lipid and lipoproteins in the general population.

Conclusions—in 4 studies including 66,831 participants and 13,642 cases with ischemic vascular events, heterozygosity for ABCC6 R1141X did not associate with risk of IHD, myocardial infarction, ICVD, or ischemic stroke. (Circ Cardiovasc Genet. 2011;4:534-541.)

Key Words: genetics ■ epidemiology ■ ischemia ■ cardiovascular diseases ■ hypertension

Pseudoxanthoma elasticum (PXE) is a rare recessive mendelian disease associated with a substantially increased risk of ischemic heart disease (IHD).1 Patients are either homozygous or compound heterozygous for loss-of-function mutations in the causative gene, ATP-Binding Cassette Transporter C6 (ABCC6).2–4 The exact biological function of the ABCC6 protein is unknown; however, widespread calcification of elastic fibers particularly affecting the skin, retina, and arteries are hallmarks of PXE.1 The cardiovascular manifestations include hypertension, cardiac failure, angina pectoris, intermittent claudication, and myocardial infarction (MI), mimicking more common heart disease.5,6 The most common recurrent PXE mutation, R1141X (rs72653706), accounts for more than 30% of all PXE mutations in the homozygous or compound heterozygous state in Caucasians.1 Case-control studies of this mutation have detected 4- to 11-fold increased risk of IHD in heterozygotes7,8. Because these studies suggest a risk of IHD comparable or larger than the risk associated with low-density lipoprotein receptor (LDLR) or apolipoprotein B (APOB) mutations, leading to familial hypercholesterolemia, and because R1141X is 3 times more common than LDLR and APOB mutations, this specific mutation is biologically well suited to test in the general population.9,10 We tested the hypothesis that heterozygosity for ABCC6 R1141X associated with ischemic vascular disease (IVD).

Clinical Perspective on p 541

First, we tested whether heterozygosity for R1141X associated with increased risk of IVD in the general population. Second, we tested whether R1141X heterozygotes were more likely to develop premature IHD compared with noncarriers. Finally, we tested whether R1141X genotype associated with...
levels of inflammatory markers (high-sensitivity C-reactive protein (hsCRP) and fibrinogen), with systolic or diastolic blood pressure or with lipids and lipoproteins, all risk factors for IVD. For these purposes, we genotyped the R1141X mutation in 10,276 participants from the Copenhagen City Heart Study (CCHS), in 45,603 participants from the Copenhagen General Population Study (CGPS), and in, respectively, 9,702 and 1,250 individuals in 2 case-control studies, the Copenhagen Ischemic Heart Disease Study and the Copenhagen Carotid Stroke Study.

Methods

Study Cohorts

We studied 4 independent cohorts of white people of Danish descent, 2 general population studies, and 2 case-control studies. These studies were defined so that no person appears in more than 1 of the 4 analyses groups, thus permitting independent confirmation of the findings in each study. Studies were approved by institutional review boards and Danish ethical committees (KF V.100.2039/91, KF 01–144/01, KF 01–062/94, KF 01–375/94, KA 99039, Copenhagen and Frederiksborg committee; and KA 93125 and KA 99039, Copenhagen County committee) and conducted according to the Declaration of Helsinki. Informed consent was obtained from all participants.

The Copenhagen City Heart Study

This is a prospective study of the general population initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003.11–13 Individuals were randomly selected based on 1-year age strata with 4851 control subjects free of IVD from the CGPS. R1141X genotype was determined in 10,385 participants attending the 1991 to 1994 and/or 2001 to 2003 examinations. We excluded 109 individuals with IVD before study entry, leaving 10,276 for all further analyses. Of these, 1985 had IHD and 989 had ischemic cerebrovascular disease (ICVD). Follow-up started at study entry and ended at occurrence of event or May 9, 2009, whichever came first. Median follow-up time was 25 years and was 100% complete, that is, none were lost to follow-up.

The Copenhagen General Population Study

This is a cross-sectional study of the general population initiated in 2003 with ongoing enrolment.11–14 Participants were recruited from the Danish general population and examined as in the CCHS. We included 45,603 participants in the present study. Of these, 3738 had IHD and 2335 had ICVD; 4851 additional participants were used as control subjects for the Copenhagen Ischemic Heart Disease Study (CIHDS), and 625 additional participants were used as control subjects for the Copenhagen Carotid Stroke Study (CCSS).

The Copenhagen Ischemic Heart Disease Study

This case-control study comprises 4851 patients from the greater Copenhagen area referred for coronary angiography to Copenhagen University Hospital initiated in 1994 and still recruiting. Experienced neurologists and vascular surgeons diagnosed ICVD together with at least 50% stenosis of a carotid artery. Hemorrhage was excluded on computed tomography scan. The 625 ICVD cases were matched on sex and 1-year age strata with 625 control subjects free of IVD from the CGPS.

End Points

In all studies, information on diagnoses of IHD (World Health Organization; International Classification of Disease, 8th edition, I10–I15; 3rd edition, I10–I15) and ICVD (World Health Organization; International Classification of Disease, 8th edition, I60–I69, G45). In the CCHS, for each person registered with ICVD, hospital records were requested. Experienced neurologists reviewed all potential cases. Possible stroke events (hospitalized as well as nonhospitalized) were validated using the World Health Organization definition of stroke: an acute disturbance of focal or global cerebral function with symptoms lasting longer than 24 hours or leading to death with presumably no other reasons than of vascular origin. To distinguish between infarction (IS), intracerebral hemorrhage, and subarachnoid hemorrhage, either a CT scan or an MRI scan, autopsy, spinal fluid examination, or surgical description was necessary. The event was diagnosed as IS if the scan did not visualize an infarction or hemorrhage but the person had symptoms that met the criteria of the stroke definition. The diagnosis of stroke was not applied in cases in which a scan revealed signs of prior cerebrovascular disease but without history of any symptoms. The diagnostic criteria for ICVD were IS, transient ischemic attack (focal neurological symptoms lasting less than 24 hours), or amaurosis fugax (transient blindness on one eye only). Cardioembolic strokes were included in the IS diagnosis.

Information on a diagnosis of atrial fibrillation (World Health Organization International Classification of Diseases, 8th edition, I40–I48; 10th edition, I48.9) was collected by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry.

Genotyping

The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc, Foster City, CA) was used to genotype the R1141X mutation. TaqMan-based assays were used. Each run included a positive control. Due to 2 rounds of reruns, call rates for genotypes were above 99.9%. Heterozygosity for the R1141X mutation was verified by DNA sequencing in CCHS, CIHDS, and CCSS. In CGPS, heterozygosity was verified by running the TaqMan-based assay twice. Concordance was 100%.

Biochemical Analyses

Total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and fibrinogen were measured using standard hospital assays (Boehringer Mannheim GmbH, Mannheim, Germany or Konelab, Helsinki, Finland). LDL cholesterol was calculated using the Friedewald equation16 if triglycerides were ≤4 mmol/L but measured directly at higher triglyceride levels (Thermo Fisher Scientific, Waltham, MA). High-sensitivity CRP was measured by turbidimetry or nephelometry (DAKO, Glostrup, Denmark, or Dade Behring, Deerfield, IL).14

Other Covariates

Diabetes mellitus, smoking, antihypertensive therapy, physical inactivity, alcohol, and lipid-lowering therapy were dichotomized and
The multifactorially adjusted hazard ratios for IHD, MI, ICVD, or IS as a function of R1141X genotype in the CCHS (carrier frequency, 0.6%), 265 in the CGPS (0.6%), 27 in the CIHDS (0.6%), and 4 in the CCSS (0.6%); none were homozygous. Genotype distributions did not deviate from Hardy-Weinberg equilibrium (P = 0.53–0.94).

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The multifactorially adjusted hazard ratios for IHD, MI, ICVD, or IS as a function of R1141X genotype in the CCHS...
did not differ from 1.0 at a \( P \) level /H11021 0.05 (Figure 1, left panel). This lack of association between genotype and IVD was confirmed in the CGPS (Figure 1, middle panel), the CIHDS (Figure 1, upper right panel), and the CCSS (Figure 1, lower right panel). When adjusted for age and sex only, or when unconditional logistic regression was performed in case-control studies instead of conditional models, results were similar.

Table 2. Characteristics of Subjects by Study and Ischemic Cerebrovascular Disease Status

<table>
<thead>
<tr>
<th>Study</th>
<th>No Event</th>
<th>Ischemic Cerebrovascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of subjects</td>
</tr>
<tr>
<td>Copenhagen City Heart Study</td>
<td>9287</td>
<td>969</td>
</tr>
<tr>
<td>Copenhagen General Population Study</td>
<td>5224/4063</td>
<td>518/471</td>
</tr>
<tr>
<td>Copenhagen Carotid Stroke Study</td>
<td>81 (74–90)</td>
<td>86 (78–94)</td>
</tr>
<tr>
<td></td>
<td>25.8 (22.4–27.8)</td>
<td>26.3 (23.5–28.8)</td>
</tr>
<tr>
<td>Physical inactivity, %</td>
<td>63</td>
<td>71</td>
</tr>
<tr>
<td>Alcohol consumption &gt;2 wk, %</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.9 (5.1–6.8)</td>
<td>6.4 (5.7–7.2)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.5 (1.0–2.1)</td>
<td>1.8 (1.3–2.5)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.6 (2.8–4.4)</td>
<td>4.0 (3.2–4.7)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.5 (1.2–1.8)</td>
<td>1.5 (1.2–1.9)</td>
</tr>
<tr>
<td>Lipid-lowering medication, %</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.8 (22.4–27.8)</td>
<td>26.3 (23.5–28.8)</td>
</tr>
</tbody>
</table>

All continuous traits are given as median and interquartile range. All categorical variables are shown in numbers and percentages. Diabetes mellitus, smoking, antihypertensive medication, physical inactivity, alcohol, and lipid-lowering therapy were dichotomized and defined as diabetes (self-reported disease, use of insulin, use of oral hypoglycemic drugs, and/or nonfasting plasma glucose /H11022 11 mmol/L), smoking (current), antihypertensive medication (daily use of antihypertensive drugs), physical inactivity (fraction of individuals with less than 2 to 4 hours per week of light physical activity at leisure time), alcohol consumption (individuals with a consumption of beer, wine, or spirits at least twice weekly), and lipid-lowering medication (daily use of lipid-lowering drugs).

*Individuals on antihypertensive medication were excluded in the analysis. NA indicates not available.

Figure 1. Risk of ischemic heart disease (IHD), myocardial infarction (MI), ischemic cerebrovascular disease (ICVD), and ischemic stroke (IS) as a function of R1141X genotype in The Copenhagen City Heart Study (CCHS), The Copenhagen General Population Study (CGPS), The Copenhagen Ischemic Heart Disease Study (CIHDS), and The Copenhagen Carotid Stroke Study (CCSS). Right panel, CIHDS is shown above the break, and CCSS is shown below. CC indicates noncarriers; CT, R1141X heterozygotes. Hazard ratios and odds ratios were multifactorially adjusted for age, sex, diabetes, smoking, and antihypertensive medication.
Figure 2. Plasma levels of high-sensitivity C-reactive protein (hsCRP), fibrinogen, systolic and diastolic blood pressure (BP), total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides as a function of R1141X genotype. Data are from the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS). We excluded participants with known ischemic heart or cerebrovascular disease and individuals who were receiving antihypertensive (analyses of systolic and diastolic BP) or lipid-lowering therapy (analyses of hsCRP, fibrinogen, and lipids and lipoproteins). All covariates were adjusted for age, sex, body mass index, and smoking. CC indicates noncarriers; CT, R1141X heterozygotes. Boxes represent median (horizontal line) and interquartile ranges; whiskers represent 5th and 95th percentiles. Probability values by Mann-Whitney U test.
To examine whether R1141X genotype interacted with age on risk, we stratified participants in those ≥55 and <55 years of age to obtain statistically valid groups. These analyses were performed in the 2 general population samples, CCHS and CGPS, for the largest end point, IHD. In these 2 analyses, R1141X genotype did not interact with age on risk of IHD (probability value for interaction: CCHS 0.76, CGPS 0.47).

Inflammatory Markers, Blood Pressure, and Lipid Levels

To examine whether R1141X genotype was associated with an exaggerated inflammatory response, increased blood pressure, or an altered lipid profile, we determined the association of R1141X genotype with plasma levels of 2 inflammatory markers (hsCRP and fibrinogen), systolic and diastolic blood pressure, and plasma lipid and lipoprotein levels (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) in participants in the CCHS and the CGPS (Figure 2). Association tests were performed only in participants without ischemic vascular events because elevated plasma levels of hsCRP and fibrinogen and increased blood pressure as well as altered lipid and lipoprotein levels are associated with increased risk of ischemic vascular events per se.14,19,20 R1141X genotype did not associate with plasma levels of hsCRP or fibrinogen, systolic or diastolic blood pressure, or plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides (Figure 2, probability values, 0.34–1.00).

Meta-Analyses

A meta-analysis including 5 independent studies on risk of IHD as a function of R1141X genotype with a total of 11 376 cases and 54 992 control subjects resulted in overall random- and fixed-effects ORs of 1.54 (95% confidence interval [CI], 0.87–2.72) and 1.30 (95% CI, 1.00–1.69), respectively, with an F of 70% (P for heterogeneity=0.01) (Figure 3, upper panel; the CCHS, the CGPS, the CIHDS, and the studies conducted by Trip MD et al7 and Köblös G et al8). A meta-analysis comprising the studies from the present report with a total of 10 574 cases and 53 186 control subjects resulted in overall random and fixed effects ORs of 1.08 (0.81–1.44) and 1.07 (95% CI, 0.80–1.43), respectively, with an F of 0% (P for heterogeneity=0.63) (Figure 3, lower panel: the CCHS, the CGPS, and the CIHDS).

Discussion

The principal finding of this study is that heterozygosity for the R1141X mutation in ABCC6 is not associated with increased risk of IHD, MI, ICVD, or IS. The absence of an association with IVD was consistently observed in four large studies, including 2 studies of the general population and 2 case-control studies, comprising more than 13 600 cases with IVD and more than 53 000 control subjects.

This is the largest study to date of heterozygous carriers of the most common PXE-causing mutation in Caucasians, accounting for more than 30% of all recurrent mutations. The risk of IHD in heterozygotes for the R1141X mutation has been reported in 2 previous case-control studies including,
Importantly, the primary aim with the present study was not to detect specific disease associated SNPs or haplotypes in the \textit{ABCC6} gene but to evaluate whether the most common disease causing mutation in PXE, in the heterozygous state conferred any PXE-associated, attenuated phenotypes in the general population, a question that could not be answered by addressing common \textit{ABCC6} variants.

Even though this study is performed in large, well-characterized cohorts of the general population and in large patient cohorts, our study has limitations. Each of the individual 4 studies has limitations and potential biases that differ from study to study owing to their different designs. Despite this, the results of the 4 studies were similar. Further, the results for ICVD and IS were not confounded by cardioembolic stroke caused by atrial fibrillation, as adjustment for atrial fibrillation did not alter the primary results. Finally, because we studied Caucasians only, our results may not necessarily apply to other ethnic groups, although PXE has been identified among most ethnicities. R1141X is the major PXE-causing mutation in Europeans possibly representing a founder mutation, whereas other \textit{ABCC6} mutations may be more common in other ethnic groups.

In conclusion, our results show that in the Danish general population, heterozygosity for R1141X, the most common PXE-causing mutation in Caucasians, does not associate with ischemic vascular disease, as previously suggested in moderately sized case-control studies in Dutch and Hungarian populations. This suggests that 1 functional \textit{ABCC6} allele is sufficient to retain normal vasculature in Caucasians in the Danish general population.

\textbf{Acknowledgments}

We thank Mette Refstrup, Karen Aagaard Hansen, and Christina Dam for their persistent attention to the details of the large-scale genotyping. We are indebted to the staff and participants of the Copenhagen City Heart Study, the Copenhagen General Population Study, and the participants in the case-control studies for their important contributions.

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\textbf{Disclosures}

None.

\textbf{References}


Patients with the rare disease pseudoxanthoma elasticum (PXE), caused by homozygosity for loss-of-function mutations in the gene ABCC6, have a substantially increased risk of ischemic heart disease caused by calcification of the vessels. We tested the hypothesis that heterozygosity for R1141X, the most frequent PXE-causing mutation in Caucasians, associated with risk of ischemic vascular disease in the general population, as previous case-control studies suggested 4- to 11-fold risk of ischemic heart disease in heterozygotes. The principal finding of this study is that heterozygosity for the R1141X mutation in ABCC6 is not associated with increased risk of ischemic vascular disease. This was consistently observed in 4 large studies, including 2 studies of the general population and 2 case-control studies, comprising more than 13 600 cases with ischemic vascular disease and more than 53 000 control subjects. Furthermore, we found no association between R1141X genotype and a moderate intermediate PXE phenotype as reflected in increased levels of inflammatory markers or increased blood pressure. Because previous smaller case-control studies suggested that heterozygosity for R1141X conferred a risk of ischemic heart disease comparable or larger than the risk associated with low density lipoprotein receptor (LDLR) or apolipoprotein B (APOB) mutations, leading to familial hypercholesterolemia, and because R1141X is 3 times more common than LDLR and APOB mutations, this hypothesis was clinically important to test. In contrast to the previous case-control studies, we found no association between R1141X in ABCC6 and risk of ischemic vascular disease in 4 large studies comprising more than 66 000 participants.
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