Conclusions—Genetic risk score improves risk prediction of CHD and helps to identify individuals at high risk for the first CHD event. Genetic screening for individuals at intermediate cardiovascular risk could help to prevent future cases through better targeting of statins. (Arterioscler Thromb Vasc Biol. 2013;33:2261-2266.)

Objective—Genome-wide association studies have identified several genetic variants associated with coronary heart disease (CHD). The aim of this study was to evaluate the genetic risk discrimination and reclassification and apply the results for a 2-stage population risk screening strategy for CHD.

Approach and Results—We genotyped 28 genetic variants in 24,124 participants in 4 Finnish population-based, prospective cohorts (recruitment years 1992–2002). We constructed a multilocus genetic risk score and evaluated its association with incident cardiovascular disease events. During the median follow-up time of 12 years (interquartile range 8.75–15.25 years), we observed 1093 CHD, 1552 cardiovascular disease, and 731 acute coronary syndrome events. Adding genetic information to conventional risk factors and family history improved risk discrimination of CHD (C-index 0.856 versus 0.851; P=0.0002) and other end points (cardiovascular disease: C-index 0.840 versus 0.837, P=0.0004; acute coronary syndrome: C-index 0.859 versus 0.855, P=0.001). In a standard population of 100,000 individuals, additional genetic screening of subjects at intermediate risk for CHD would reclassify 2144 subjects (12%) into high-risk category. Statin allocation for these subjects is estimated to prevent 135 CHD cases over 14 years. Similar results were obtained by external validation, where the effects were estimated from a training data set and applied for a test data set.

Coronary heart disease (CHD) is a complex disorder with the risk modified by both environmental and genetic factors. At present, the established risk factors, such as high cholesterol and blood pressure, explain only a fraction of the variability in disease risk. This has motivated a search for new predictors, including genetic markers. Several genome-wide association studies have identified many novel genetic susceptibility loci for CHD.1-3 Although causal variants and biological function are still unknown for many of the loci, their potential for better identifying the high-risk individuals has been studied. Genetic risk scores (GRSs) based on the subsets of the most strongly associated single-nucleotide polymorphisms (SNPs) in the identified loci have been associated with future CHD events in samples of European origin,4,5 but studies so far have shown little or no additional value for GRS’ ability to predict future cases of CHD over the traditional risk factors.

See accompanying article on page 2049

Recently, the Emerging Risk Factors Collaboration8,9 has evaluated lipid-related and inflammatory markers by their ability to improve risk classification in a 2-stage population screening strategy. In this approach, a population of 100,000 individuals is first screened for the traditional cardiovascular risk factors. In the second stage, additional screening based on a novel risk marker is conducted for the subjects at the intermediate-risk category (10-year risk, 10%–20%). The guidelines from National Institute for Health and Care Excellence10 and ATP-III11 among others recommend that statin treatment should be allocated for the individuals with 10-year absolute risk of cardiovascular disease (CVD) >20%. Thus, identification of individuals at the intermediate-risk category, who would benefit from long term statin medication, could have both clinical and population health benefits.

In this study, we genotyped 28 previously identified genetic risk variants for CHD2,3,12,13 in 4 Finnish prospective cohorts (n=24,124) with up to 19 years of follow-up. We set out to evaluate the genetic risk discrimination of CHD, acute coronary syndrome (ACS), and combined CHD and stroke events (CVD), and estimate the improved risk classification of CHD in a 2-stage population screening strategy.

Materials and Methods

Materials and Methods are available in the online-only Supplement.
Results

Background Characteristics of Study Cohorts

Characteristics of study cohorts are shown in Table 1. In total, 24,124 subjects from FINRISK 1992, FINRISK 1997, FINRISK 2002, and Health 2000 cohorts were included in the analysis. We observed 1093 CHD (5%), 1552 CVD (6%), and 731 ACS (3%) cases during the median follow-up time of 12 years (interquartile range 8.75–15.25 years).

Association Results

When tested individually, 5 loci were associated with all cardiovascular end points: rs6725887 (reported locus: WDR12), rs12526453 (PHACTR1), rs4977574 (CDKN2A/B, ANRIL), rs1746048 (CXCL12), and rs3825807 (ADAMTS7). In total, 13 loci were significantly associated with ≥1 of the end points. (Table II in the online-only Data Supplement).

The GRS was associated with all cardiovascular end points (Table 2). Subjects in the highest 10% of 28-SNP GRS had 2.07-fold (95% confidence interval [CI], 1.68–2.56; P=9.8×10−14) increased risk for CHD, when compared with the subjects in the middle 20%. When GRS was constructed by using only 13 SNPs that have been identified in the first phase of genome-wide studies,1 the corresponding risk for 13-SNP GRS was 1.55 (95% CI, 1.26–1.91; P=4.7×10−11).

When dividing the risk score into deciles, the highest GRS group was clearly distinguishable from the other groups, especially in CHD and CVD events (Figure 1 and Figure I in the online-only Data Supplement). The deviation from the linear risk function was observed for the highest decile in CHD (additional HR over linear risk, 1.38; 95% CI, 1.12–1.72; P=0.003) and CVD (HR, 1.39; 95% CI, 1.15–1.67; P=0.0006), but not for ACS (HR, 1.16; 95% CI, 0.89–1.51; P=0.27). The nonlinearity does not seem to be driven by the weights in the GRS because similar nonlinear risk function was observed also for unweighted GRS (Figure II in the online-only Data Supplement).

In FINRISK studies, family history of CVD was an independent risk factor for all events. Adjusting for the GRS slightly diminished the effects of family history: The estimated risk decreased from 1.46 (95% CI, 1.27–1.67) to 1.43 (95% CI, 1.25–1.64) for CHD, from 1.37 (95% CI, 1.22–1.53) to 1.35 (95% CI, 1.21–1.51) for CVD, and from 1.45 (95% CI, 1.24–1.71) to 1.43 (95% CI, 1.21–1.68) for ACS, for the models with and without the GRS, respectively. In comparison, the GRS effects (per SD of GRS) changed from 1.29 (95% CI, 1.20–1.38) to 1.28 (95% CI, 1.19–1.37) for CHD, from 1.19 (95% CI, 1.12–1.26) to 1.18 (95% CI, 1.12–1.25) for CVD, and from 1.30 (95% CI, 1.19–1.40) to 1.29 (95% CI, 1.19–1.40) for ACS, when family history was included into the model.

Table 1. Characteristics of Study Cohorts

<table>
<thead>
<tr>
<th></th>
<th>FINRISK 1992 (n=5104)</th>
<th>FINRISK 1997 (n=6567)</th>
<th>FINRISK 2002 (n=7330)</th>
<th>Health 2000 (n=5123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up, y</td>
<td>19</td>
<td>14</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>2287 (44.8)</td>
<td>3005 (45.8)</td>
<td>3299 (45.0)</td>
<td>2371 (46.3)</td>
</tr>
<tr>
<td>Women</td>
<td>2817 (55.2)</td>
<td>3562 (54.2)</td>
<td>4031 (55.0)</td>
<td>2752 (53.7)</td>
</tr>
<tr>
<td>Age, y*</td>
<td>43.9±11.3</td>
<td>46.8±12.9</td>
<td>47.5±13.0</td>
<td>50.0±11.7</td>
</tr>
<tr>
<td>Cholesterol, mmol/L*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.6±1.1</td>
<td>5.5±1.1</td>
<td>5.6±1.1</td>
<td>5.9±1.1</td>
</tr>
<tr>
<td>LDL</td>
<td>3.5±1.0</td>
<td>3.5±0.9</td>
<td>3.4±0.9</td>
<td>3.8±1.2</td>
</tr>
<tr>
<td>HDL</td>
<td>1.4±0.3</td>
<td>1.4±0.4</td>
<td>1.5±0.4</td>
<td>1.3±0.4</td>
</tr>
<tr>
<td>Blood pressure, mmHg*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>135.1±19.3</td>
<td>134.9±19.5</td>
<td>134.8±19.9</td>
<td>132.7±20.1</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81.1±11.9</td>
<td>82.1±11.2</td>
<td>79.0±11.4</td>
<td>82.2±11.1</td>
</tr>
<tr>
<td>Body mass index (weight/height²), kg/m²*</td>
<td>26.0±4.4</td>
<td>26.5±4.6</td>
<td>26.8±4.7</td>
<td>26.8±4.7</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>1425 (27.9)</td>
<td>1606 (24.5)</td>
<td>1928 (26.3)</td>
<td>1611 (31.4)</td>
</tr>
<tr>
<td>Antihypertensive medication, n (%)</td>
<td>443 (8.7)</td>
<td>761 (11.6)</td>
<td>979 (13.4)</td>
<td>953 (18.6)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>172 (3.4)</td>
<td>323 (4.9)</td>
<td>358 (4.9)</td>
<td>291 (5.7)</td>
</tr>
<tr>
<td>Family history of cardiovascular disease, n (%)</td>
<td>1376 (27.0)</td>
<td>2350 (37.8)</td>
<td>2426 (33.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Incident cases, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>501 (9.8)</td>
<td>499 (7.6)</td>
<td>291 (4.0)</td>
<td>261 (5.1)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>343 (6.7)</td>
<td>344 (5.2)</td>
<td>209 (2.9)</td>
<td>197 (3.8)</td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
<td>235 (4.6)</td>
<td>229 (3.5)</td>
<td>148 (2.0)</td>
<td>119 (2.3)</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; n, number of individuals; and NA, information not available.

*Mean±SD.
Risk Discrimination and Reclassification

The model including traditional risk factors had the C-index of 0.849 for CHD, 0.835 for CVD, and 0.853 for ACS. Adding family history of CVD into the model improved C-index by 0.2% \( (P=0.03 \text{ for both CHD and CVD); Figure 2} \). The GRS further improved risk discrimination of all end points over and above traditional risk factors and family history by 0.3% to 0.5% \( (P=0.0002 \text{ for CHD}; P=0.0004 \text{ for CVD}; P=0.001 \text{ for ACS; Figure 2}) \).

There were 576 incident CHD cases in the combined 14-year follow-up of FINRISK 1992 and 1997. The family history of CVD did not improve the reclassification (net reclassification improvement [NRI]=0.1%; \( P=0.40 \)). Adding the GRS into the model with traditional risk factors and family history resulted in overall NRI of 5% \( (P=0.01) \). We also observed a significant improvement in reclassification of individuals at the intermediate-risk category (clinical NRI=27%; \( P=1.1\times10^{-4} \)). Overall, 52 CHD cases (27%) and 206 noncases (20%) in the intermediate-risk group were correctly reclassified, when the GRS was added into the model (Table 3). We obtained essentially similar results for NRI in our sensitivity analysis with 2% higher categorical thresholds (Table III in the online-only Data Supplement).

Also, integrated discrimination improvement (value=0.007; \( P=4.2\times10^{-5} \)) indicated statistically significant improvement in prediction, when genetic information was added to the model. Explained relative risk was 0.43 (SE=0.02) for the model without the GRS and 0.45 (SE=0.02) with the GRS. Calibration was good for all end points (Hosmer–Lemeshow test, 0.677\( P=0.12 \)).

We assessed the potential overfitting of the models by using the FINRISK 2002 as a training set and the joint FINRISK 1992 and 1997 data as a test data set. To keep the training and test data sets comparable, we excluded Health 2000 from the analysis because it lacks the information on family history. We estimated the effects for 2 models (with and without the GRS) in the training set data and used the estimated effect sizes from the training models to predict the 14-year absolute risk in the test data. The baseline hazard was estimated from the test data. This approach resulted in essentially similar results as a method, where effect sizes were estimated directly from the test data set (Table IV in the online-only Data Supplement).

Traditional risk factor screening of 100 000 European individuals would classify 64 373 subjects into <10%, 18 223 into 10% to 20%, and 17 404 into ≥20% risk category (Figure 3). On the basis of the present guidelines, \( ^{10,11} \) only individuals in the highest risk group are eligible for lipid medication. We also classified those subjects with baseline diabetes mellitus or lipid treatment directly to the high-risk category. Thus, subjects at the intermediate-risk category (10%–20%) were assumed not to receive statin treatment. Additional GRS screening of these subjects would reclassify 3475 subjects (19%) into the low- and 2144 (12%) into the high-risk category. Of the subjects reclassified into the high-risk category, 676 were expected to experience CHD event within 14 years. Assuming that statins reduce the risk by 20%, additional GRS screening could prevent 135 (676\( \times 0.2 \)) CHD cases over 14 years. As a comparison, if statins would be randomly allocated to a comparable number of individuals predicted with intermediate risk in the absence of the GRS, screening would prevent 2.5 times more events than the random allocation of statins to a comparable number of individuals.

Discussion

We studied 4 prospective Finnish cohorts with genetic markers from 28 loci that have been associated with CHD or myocardial infarction in previous studies.\(^ {2,3,12,13} \) The GRS based on these variants was strongly associated with cardiovascular events and improved risk discrimination for all end points (C-index change =0.3%–0.5%, all \( P \) values ≤0.001). GRS also improved risk reclassification of CHD in a joint FINRISK 1992 and FINRISK 1997 analyses.
(NRI=5%, \(P=0.01\); clinical NRI=27%, \(P=1.1\times10^{-8}\)). Also integrated discrimination improvement and explained relative risk indicated improved prediction. In our clinical modeling of 100,000 individuals, targeted GRS screening of clinically relevant risk group (10%–20%) would reclassify 2144 subjects (12%) in the intermediate- to high-risk category. Statin allocation for reclassified individuals could prevent 135 CHD cases over 14 years.

Our results allow us to draw the following conclusions, which may be of clinical interest when evaluating a healthy individual’s risk for CHD. First, the GRS is associated with incident CHD with the risk >2-fold for an individual at the

![Figure 2](image-url) Changes in C-index in FINRISK cohorts when adding family history of cardiovascular disease and the genetic risk score to the model. The reference (ref) model with the traditional risk factors had the C-index of 0.849 for coronary heart disease, 0.835 for cardiovascular disease, and 0.853 for acute coronary syndrome.

### Table 3. Reclassification of Individuals in 4 Risk Categories After Addition of GRS to a Model With Traditional Risk Factors and Family History*

<table>
<thead>
<tr>
<th>Model Without GRS</th>
<th>0% to 5%</th>
<th>5% to 10%</th>
<th>10% to 20%</th>
<th>&gt;20%</th>
<th>NRI</th>
<th>Clinical NRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>79</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Events: 0.04 ((P=0.03); nonevents: 0.01)</td>
</tr>
<tr>
<td>Nonevents</td>
<td>7820</td>
<td>199</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Events: 0.15 ((P=4.6\times10^{-4}); nonevents: 0.11)</td>
</tr>
<tr>
<td>All</td>
<td>7899</td>
<td>214</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Events: 0.04 ((P=0.002); all: 0.05)</td>
</tr>
<tr>
<td>5% to 10%</td>
<td>16</td>
<td>94</td>
<td>21</td>
<td>0</td>
<td></td>
<td>Events: 0.27 ((P=3.3\times10^{-3}); all: 0.37)</td>
</tr>
<tr>
<td>Events</td>
<td>249</td>
<td>1080</td>
<td>173</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonevents</td>
<td>265</td>
<td>1174</td>
<td>194</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>265</td>
<td>1174</td>
<td>194</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% to 20%</td>
<td>0</td>
<td>22</td>
<td>122</td>
<td>52</td>
<td></td>
<td></td>
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<tr>
<td>Events</td>
<td>0</td>
<td>206</td>
<td>745</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonevents</td>
<td>0</td>
<td>228</td>
<td>867</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0</td>
<td>228</td>
<td>867</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;20%</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>186</td>
<td></td>
<td></td>
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<tr>
<td>Events</td>
<td>0</td>
<td>0</td>
<td>105</td>
<td>377</td>
<td></td>
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</tr>
<tr>
<td>Nonevents</td>
<td>0</td>
<td>0</td>
<td>127</td>
<td>563</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0</td>
<td>0</td>
<td>127</td>
<td>563</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*GRS indicates genetic risk score; and NRI, net reclassification improvement.

**Traditional risk factors include sex, total cholesterol, high-density lipoprotein–cholesterol, body mass index, systolic blood pressure, blood pressure treatment, smoking, and type 2 diabetes mellitus; age was used as the timescale in the Cox proportional hazards model.
Figure 3. Two-stage risk screening of coronary heart disease in a standard population of 100,000 subjects. *On the basis of guidelines, the subjects at intermediate-risk group (10%–20%) are assumed not to receive statin treatment. Statins are presently allocated for the subjects at ≥20% risk group. In addition, subjects with baseline lipid treatment or diabetes mellitus were assumed treated.

There are 100,000 people screened. The top 10% of GRS compared with an average subject. The risk for these individuals is higher than expected by a linear function (P=0.003) and may be underestimated by predictions based only on traditional risk factors. Second, the GRS improved risk discrimination of CHD over and above the conventional risk factors and family history, when evaluated with either discrimination or reclassification measures. Improved risk classification led to more accurate risk categorization for individuals at intermediate-risk group, which means that a substantial proportion of CHD cases was reclassified upward and noncases downward in the risk scale (0%–5%, 5%–10%, 10%–20%, >20%). Because these risk categories have been developed to guide treatment decisions, improved risk reclassification may have public health benefits. To address this, we estimated the effect of reclassification in a population level. Our data suggest that targeted GRS screening in addition to traditional risk factor screening would prevent 1 additional CHD event during the period of 14 years for every 135 people (18 223/135) screened.

Strengths of our study include a large prospective data set and accurately defined event definitions, which have been drawn from the validated population registries. The main end point of our study was CHD (n=1093), rather than more heterogeneous CVD, which has been used in some other genetic risk prediction studies that have failed to show an incremental value of the GRS. However, our estimates are comparable with the recent study that analyzed 742 CHD events and constructed the GRS with the comparable SNP set to our study. The authors observed improvements in reclassification (NRI=2.8%; P=0.031), but not in discrimination. The better statistical power attributable to the larger number of SNPs or CHD events in our study might partially explain why we observed improvement in both reclassification and discrimination. Also, genetic diversity is lower and the extent of linkage disequilibrium is higher in Finland than in other European populations, which might facilitate the study of genetic effects for complex diseases.

Our large population cohort is well suitable for clinical modeling, a concept that has been applied in 2 recent risk prediction studies. The Emerging Risk Factors Collaboration studied the added use of lipid-markers in cardiovascular risk prediction. Additional screening based on lipoprotein(a) resulted in reclassification of 555 subjects from intermediate- to high-risk category and potential prevention of 17 CVD events over 10 years. The other study by the same collaboration studied the added use of 28-SNP GRS in risk prediction is superior compared with these other novel cardiovascular risk markers.

Our results should also be interpreted in the context of potential limitations of our study. Although our 28-SNP marker panel consists of lead SNPs from associated loci, we are only catching a fraction of all genetic risk variation for CHD. In our reclassification analyses, we combined 2 study cohorts and estimated 14-year CHD risk. However, established risk categories are usually applied for a 10-year time frame and may not be directly applicable to our extended follow-up period. We, however, have addressed this issue by performing a sensitivity analysis with 2% higher risk thresholds. Also, we assumed that all participants eligible for statin treatment would receive them, which might overestimate the benefits of 2-stage risk screening. In practice, compliance to treatment might not be complete. Finally, this study was conducted using a Finnish population sample, and the generalizability of these results to other populations, especially to those of non-European ethnicity, needs to be confirmed.

In conclusion, GRS improves CHD risk discrimination and reclassification over and above traditional risk factors and family history. Additional GRS screening of individuals at intermediate cardiovascular risk could help to prevent future cases through more accurate statin allocation. The clinical, economical, and practical use of the genetic testing needs to be further tested.

Sources of Funding
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Disclosures
Dr Ripatti was supported by the Academy of Finland Center of Excellence in Complex Disease Genetics (no.s 213506 and 129680), Academy of Finland (no. 251217), the Finnish foundation for Cardiovascular Research and the Sigrid Juselius Foundation. Dr Salomaa was supported by grant numbers 130635 and 129494 from the Academy of Finland and the Finnish Foundation for Cardiovascular Research. Authors declare that they have no competing interest. E. Tikkanen had full access to all data in the study and...
takes responsibility for the integrity of the data and the accuracy of the data analysis. The other authors report no conflicts.

**References**


**Significance**

The majority of the cardiovascular events occur within a population who are not classified as high risk, on the basis of the traditional risk factors. This has motivated the search for new potential risk markers. Genome-wide association studies have identified several common single-nucleotide polymorphisms associated with coronary heart disease in case-control data sets. In this study, we show that the genetic risk score of these variants improves the risk discrimination and reclassification of coronary heart disease over and above traditional risk factors and family history in a prospective study setting. We applied the reclassification results into standard European population of 100,000 individuals and showed that targeted genetic screening of individuals at intermediate risk (10%–20%) could prevent 1 additional coronary heart disease event during the period of 14 years for every 135 people (18223/135) screened. The clinical, economical, and practical use of genetic screening of individuals in the intermediate risk for coronary heart disease needs to be further tested.
Genetic Risk Prediction and a 2-Stage Risk Screening Strategy for Coronary Heart Disease
Emmi Tikkanen, Aki S. Havulinna, Aarno Palotie, Veikko Salomaa and Samuli Ripatti

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Supplement Material

This file includes

1. Supplementary Table I. Single nucleotide polymorphisms (SNPs) associated with coronary heart disease
2. Supplementary Table II. Association results for single nucleotide polymorphisms (SNPs) and cardiovascular events
3. Supplementary Table III. Reclassification of individuals in four risk categories after addition of genetic risk score (GRS) to a model with traditional risk factors – sensitivity analysis
4. Supplementary Table IV. Assessment of over-fitting in discrimination and reclassification measures
5. Supplementary Figure I. Genetic risk score deciles and risk for cardiovascular disease (CVD) and acute coronary syndrome (ACS)
6. Supplementary Figure II. Risk allele count and risk for coronary heart disease (CHD), acute coronary syndrome (ACS) and cardiovascular disease (CVD)
7. References
### Supplementary Table I. Single nucleotide polymorphisms (SNPs) associated with coronary heart disease

<table>
<thead>
<tr>
<th>SNP</th>
<th>Region</th>
<th>Reported Gene(s)</th>
<th>Risk allele</th>
<th>Risk allele freq. in controls</th>
<th>Weight†</th>
<th>Reference</th>
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<td>rs646776</td>
<td>1p13.3</td>
<td>CELSR2, PSRC1, SORT1</td>
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<td>WDR12</td>
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</tr>
<tr>
<td>rs12526453</td>
<td>6p24.1</td>
<td>PHACTR1</td>
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<tr>
<td>rs4977574</td>
<td>9p21.3</td>
<td>CDKN2A/B, ANRIL</td>
<td>G</td>
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<td>1.29</td>
<td>2</td>
</tr>
<tr>
<td>rs1746048</td>
<td>10q11.21</td>
<td>CXCL12</td>
<td>C</td>
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<td>1.07</td>
<td>2</td>
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<tr>
<td>rs1122608</td>
<td>19p13.2</td>
<td>LDLR</td>
<td>G</td>
<td>0.79</td>
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</tr>
<tr>
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<td>MRPS6</td>
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<td>12q24.31</td>
<td>HNFA1</td>
<td>T</td>
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<td>1.08</td>
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</tr>
<tr>
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<td>PPAP2B</td>
<td>A</td>
<td>0.89</td>
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<td>6p21.31</td>
<td>ANKS1A</td>
<td>G</td>
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<td>7q32.2</td>
<td>ZC3HC1</td>
<td>C</td>
<td>0.67</td>
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<tr>
<td>rs579459</td>
<td>9q34.2</td>
<td>ABO</td>
<td>C</td>
<td>0.22</td>
<td>1.10</td>
<td>2</td>
</tr>
<tr>
<td>rs12413409</td>
<td>10q24.32</td>
<td>CYP17A1, CNNM2, NT5C2</td>
<td>G</td>
<td>0.92</td>
<td>1.12</td>
<td>2</td>
</tr>
<tr>
<td>rs964184</td>
<td>11q23.3</td>
<td>ZNF259, APOA5-A4-C3-A1</td>
<td>G</td>
<td>0.14</td>
<td>1.13</td>
<td>2</td>
</tr>
<tr>
<td>rs4773144</td>
<td>13q34</td>
<td>COL4A1, COL4A2</td>
<td>G</td>
<td>0.40</td>
<td>1.07</td>
<td>2</td>
</tr>
<tr>
<td>rs2895811</td>
<td>14q32.2</td>
<td>HHIPL1</td>
<td>C</td>
<td>0.42</td>
<td>1.07</td>
<td>2</td>
</tr>
<tr>
<td>rs3825807</td>
<td>15q25.1</td>
<td>ADAMTS7</td>
<td>A</td>
<td>0.65</td>
<td>1.08</td>
<td>2</td>
</tr>
<tr>
<td>rs12936587</td>
<td>17p11.2</td>
<td>RASD1, SMCR3, PEMT</td>
<td>G</td>
<td>0.65</td>
<td>1.07</td>
<td>2</td>
</tr>
<tr>
<td>rs216172</td>
<td>17p13.3</td>
<td>SMG6, SRR</td>
<td>C</td>
<td>0.35</td>
<td>1.07</td>
<td>2</td>
</tr>
<tr>
<td>SNP</td>
<td>Region</td>
<td>Reported Gene(s)</td>
<td>Risk allele</td>
<td>Risk allele freq. in controls</td>
<td>Weight†</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>------------------</td>
<td>-------------</td>
<td>------------------------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>rs46522</td>
<td>17q21.32</td>
<td>UBE2Z, GIP, ATP5G1, SNF8</td>
<td>T</td>
<td>0.55</td>
<td>0.53</td>
<td>1.06</td>
</tr>
<tr>
<td>rs1412444</td>
<td>10q23.31</td>
<td>LIPA</td>
<td>T</td>
<td>0.42</td>
<td>0.42</td>
<td>1.09</td>
</tr>
<tr>
<td>rs4380028</td>
<td>15q25.1</td>
<td>ADAMTS7-MORF4L1</td>
<td>C</td>
<td>0.70</td>
<td>0.65</td>
<td>1.07</td>
</tr>
<tr>
<td>rs10953541</td>
<td>7q22.3</td>
<td>NR</td>
<td>C</td>
<td>0.74</td>
<td>0.80</td>
<td>1.08</td>
</tr>
</tbody>
</table>

† Odds ratios from the reference studies were used as weights for the risk allele counts to generate the genetic risk score.
### Supplementary Table II. Association results for single nucleotide polymorphisms (SNPs) and cardiovascular events

<table>
<thead>
<tr>
<th>SNP</th>
<th>Region</th>
<th>Reported Gene(s)</th>
<th>Risk allele</th>
<th>Coronary heart disease</th>
<th>Acute coronary syndrome</th>
<th>Cardiovascular disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HR (95% CI)</td>
<td>P value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>rs646776</td>
<td>1p13.3</td>
<td>CELSR2, PSRC1, SORT1</td>
<td>T</td>
<td>1.05 (0.94, 1.17)</td>
<td>0.37</td>
<td>1.02 (0.90, 1.15)</td>
</tr>
<tr>
<td>rs11206510</td>
<td>1p32.3</td>
<td>PCSK9</td>
<td>T</td>
<td>1.04 (0.93, 1.17)</td>
<td>0.51</td>
<td>1.07 (0.93, 1.23)</td>
</tr>
<tr>
<td>rs17465637</td>
<td>1q41</td>
<td>MIA3</td>
<td>C</td>
<td>1.04 (0.94, 1.15)</td>
<td>0.41</td>
<td>1.05 (0.93, 1.19)</td>
</tr>
<tr>
<td>rs6725887</td>
<td>2q33.1</td>
<td>WDR12</td>
<td>C</td>
<td>1.26 (1.11, 1.43)</td>
<td>0.0003</td>
<td>1.21 (1.04, 1.42)</td>
</tr>
<tr>
<td>rs2306374</td>
<td>3q22.3</td>
<td>MRAS</td>
<td>C</td>
<td>1.13 (1.04, 1.23)</td>
<td>0.004</td>
<td>1.19 (1.07, 1.32)</td>
</tr>
<tr>
<td>rs12526453</td>
<td>6q24.1</td>
<td>PHACTR1</td>
<td>C</td>
<td>1.43 (1.02, 2.00)</td>
<td>0.04</td>
<td>1.20 (0.77, 1.86)</td>
</tr>
<tr>
<td>rs3798220</td>
<td>6q25.3</td>
<td>LPA</td>
<td>C</td>
<td>1.19 (1.09, 1.29)</td>
<td>5.4×10⁻⁵</td>
<td>1.16 (1.04, 1.28)</td>
</tr>
<tr>
<td>rs1746048</td>
<td>10q11.2</td>
<td>CXCL12</td>
<td>C</td>
<td>1.31 (1.16, 1.49)</td>
<td>2.2×10⁻⁵</td>
<td>1.33 (1.14, 1.54)</td>
</tr>
<tr>
<td>rs3184504</td>
<td>12q24.1</td>
<td>SH2B3</td>
<td>T</td>
<td>1.02 (0.94, 1.12)</td>
<td>0.60</td>
<td>1.04 (0.94, 1.15)</td>
</tr>
<tr>
<td>rs1122608</td>
<td>19p13.2</td>
<td>LDLR</td>
<td>G</td>
<td>1.10 (0.98, 1.22)</td>
<td>0.10</td>
<td>1.12 (0.98, 1.27)</td>
</tr>
<tr>
<td>rs9982601</td>
<td>21q22.1</td>
<td>MRPS6</td>
<td>T</td>
<td>1.18 (1.05, 1.33)</td>
<td>0.005</td>
<td>1.17 (1.02, 1.35)</td>
</tr>
<tr>
<td>rs2259816</td>
<td>12q24.3</td>
<td>HNF1A</td>
<td>T</td>
<td>1.01 (0.93, 1.10)</td>
<td>0.83</td>
<td>1.04 (0.94, 1.16)</td>
</tr>
<tr>
<td>rs17114036</td>
<td>1p32.2</td>
<td>PPAP2B</td>
<td>A</td>
<td>1.06 (0.92, 1.21)</td>
<td>0.43</td>
<td>1.02 (0.87, 1.20)</td>
</tr>
<tr>
<td>rs17609940</td>
<td>6p21.31</td>
<td>ANKS1A</td>
<td>G</td>
<td>1.02 (0.92, 1.14)</td>
<td>0.72</td>
<td>1.05 (0.92, 1.20)</td>
</tr>
<tr>
<td>rs11556924</td>
<td>7q32.2</td>
<td>ZC3HC1</td>
<td>C</td>
<td>1.01 (0.92, 1.11)</td>
<td>0.80</td>
<td>1.00 (0.90, 1.12)</td>
</tr>
<tr>
<td>rs579459</td>
<td>9q34.2</td>
<td>ABO</td>
<td>C</td>
<td>1.10 (0.99, 1.21)</td>
<td>0.07</td>
<td>1.13 (1.00, 1.27)</td>
</tr>
<tr>
<td>rs12413409</td>
<td>10q24.3</td>
<td>CYP17A1, CNNM2, NT5C2</td>
<td>G</td>
<td>1.04 (0.89, 1.21)</td>
<td>0.60</td>
<td>1.08 (0.90, 1.30)</td>
</tr>
<tr>
<td>SNP</td>
<td>Region</td>
<td>Reported Gene(s)</td>
<td>Risk allele</td>
<td>Coronary heart disease</td>
<td>Acute coronary syndrome</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
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<tr>
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<td></td>
<td>HR (95% CI)</td>
<td>P value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>rs964184</td>
<td>11q23.3</td>
<td>ZNF259, APOA5-A4-C3-A1</td>
<td>G</td>
<td>1.02 (0.90, 1.15)</td>
<td>0.78</td>
<td>1.01 (0.88, 1.17)</td>
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<tr>
<td>rs4773144</td>
<td>13q34</td>
<td>COL4A1, COL4A2</td>
<td>G</td>
<td>1.10 (1.01, 1.20)</td>
<td>0.03</td>
<td>1.07 (0.96, 1.18)</td>
</tr>
<tr>
<td>rs2895811</td>
<td>14q32.2</td>
<td>HHIPL1</td>
<td>C</td>
<td>1.12 (1.03, 1.22)</td>
<td>0.009</td>
<td>1.10 (0.99, 1.22)</td>
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<tr>
<td>rs3825807</td>
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<td>ADAMTS7</td>
<td>A</td>
<td>1.13 (1.03, 1.23)</td>
<td>0.008</td>
<td>1.12 (1.01, 1.25)</td>
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<tr>
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<td>RASD1, SMCR3, PEMT</td>
<td>G</td>
<td>1.11 (1.02, 1.22)</td>
<td>0.02</td>
<td>1.05 (0.94, 1.16)</td>
</tr>
<tr>
<td>rs216172</td>
<td>17p13.3</td>
<td>SMG6, SRR</td>
<td>C</td>
<td>1.06 (0.97, 1.16)</td>
<td>0.19</td>
<td>1.04 (0.93, 1.15)</td>
</tr>
<tr>
<td>rs46522</td>
<td>17q21.3</td>
<td>UBE2Z, GIP, ATP5G1, SNF8</td>
<td>T</td>
<td>0.97 (0.89, 1.06)</td>
<td>0.49</td>
<td>0.98 (0.88, 1.08)</td>
</tr>
<tr>
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<td>LIPA</td>
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<td>0.98 (0.9, 1.07)</td>
<td>0.63</td>
<td>1.00 (0.90, 1.11)</td>
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<tr>
<td>rs4380028</td>
<td>15q25.1</td>
<td>ADAMTS7-MORF4L1</td>
<td>C</td>
<td>1.12 (1.02, 1.23)</td>
<td>0.02</td>
<td>1.15 (1.02, 1.28)</td>
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<tr>
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<td>NR</td>
<td>C</td>
<td>1.03 (0.94, 1.14)</td>
<td>0.50</td>
<td>1.04 (0.92, 1.17)</td>
</tr>
</tbody>
</table>

Cox proportional hazards model adjusted for sex, total cholesterol, high-density lipoprotein (HDL) cholesterol, body mass index, systolic blood pressure, blood pressure treatment, smoking and type 2 diabetes; age was used as the timescale.

Abbreviations: HR, Hazard ratio; CI, confidence interval.
### Supplementary Table III. Reclassification of individuals in four risk categories after addition of genetic risk score (GRS) to a model with traditional risk factors* – sensitivity analysis**

<table>
<thead>
<tr>
<th>Model without GRS</th>
<th>Model with GRS</th>
<th>NRI</th>
<th>Clinical NRI</th>
</tr>
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<tr>
<td></td>
<td>0-7%</td>
<td>7-12%</td>
<td>12-22%</td>
</tr>
<tr>
<td>Events</td>
<td>121</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Nonevents</td>
<td>8564</td>
<td>197</td>
<td>0</td>
</tr>
<tr>
<td>All</td>
<td>8685</td>
<td>219</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7-12%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>15</td>
<td>77</td>
<td>23</td>
</tr>
<tr>
<td>Nonevents</td>
<td>251</td>
<td>689</td>
<td>140</td>
</tr>
<tr>
<td>All</td>
<td>266</td>
<td>766</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>12-22%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>0</td>
<td>25</td>
<td>122</td>
</tr>
<tr>
<td>Nonevents</td>
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<td>546</td>
</tr>
<tr>
<td>All</td>
<td>2</td>
<td>207</td>
<td>668</td>
</tr>
<tr>
<td></td>
<td>&gt;22%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Nonevents</td>
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<td>1</td>
<td>92</td>
</tr>
<tr>
<td>All</td>
<td>0</td>
<td>1</td>
<td>113</td>
</tr>
</tbody>
</table>

*Traditional risk factors include sex, total cholesterol, high-density lipoprotein (HDL) cholesterol, body-mass index, systolic blood pressure, blood pressure treatment, smoking and type 2 diabetes; age was used as the timescale in the Cox proportional hazards model.

** Sensitivity of the choice of categories was tested by rising category thresholds by 2%.

Abbreviations: NRI, net reclassification improvement

### Supplementary Table IV. Assessment of over-fitting in discrimination and reclassification measures

<table>
<thead>
<tr>
<th></th>
<th>Effects estimated directly from the test dataset*</th>
<th>Effects estimated from the training dataset** applied for calculating the linear predictors in the test dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-index of the model with traditional risk factors</td>
<td>0.859</td>
<td>0.855</td>
</tr>
<tr>
<td>C-index change with the addition of the GRS (P-value)</td>
<td>0.004 (P=0.007)</td>
<td>0.004 (P=0.012)</td>
</tr>
<tr>
<td>IDI (P-value)</td>
<td>0.007 (P=4.2×10⁻⁵)</td>
<td>0.009 (P=1.3×10⁻⁵)</td>
</tr>
<tr>
<td>NRI (P-value)</td>
<td>0.05 (P=0.01)</td>
<td>0.04 (P=0.05)</td>
</tr>
</tbody>
</table>

* Test dataset: combined FINRISK 1992 and 1997 dataset

** Training dataset: FINRISK 2002 dataset. To justify the comparison with FINRISK 1992 and 1997 datasets, individuals with baseline lipid medication (N=454) were removed from the analysis.

Abbreviations: FR, FINRISK; GRS, genetic risk score; IDI, integrated discrimination index; NRI, net reclassification improvement
Supplementary Figure I. Genetic risk score deciles and risk for cardiovascular disease (CVD) and acute coronary syndrome (ACS).

Supplementary Figure II. Risk allele count and risk for coronary heart disease (CHD), acute coronary syndrome (ACS) and cardiovascular disease (CVD).
References


MATERIAL AND METHODS

Study Populations

FINRISK surveys have been conducted every 5 years since 1972 to monitor the risk of chronic diseases. For each survey, a stratified random sample was selected from the 25–74 year old inhabitants in different regions in Finland. The overlap between the samples is due to a small number of individuals being randomly chosen to consecutive FINRISK surveys. In surveys 1992–2007, 98% of the non-prevalent observations are unique. The survey included a questionnaire and a clinical examination, where a blood sample was drawn. The study protocol has been described elsewhere. FINRISK surveys 1992, 1997 and 2002 were included in the current analysis.

Health 2000 was based on a stratified two-stage cluster sampling from the National Population Information system to represent the total Finnish population aged 30 years and over. Persons aged ≥ 80 years were oversampled with a sampling weight of 2. The survey included an interview about medical history, health-related lifestyle habits, and a clinical examination at which a blood sample was drawn. A detailed methodology report is available online.

During the follow-up, hospitalization and mortality data were obtained from the Finnish National Hospital Discharge Register and the Finnish National Causes-Of-Death Register. These registers cover all cardiovascular events that have led either to hospitalization or death in Finland. Cardiovascular diagnoses in these registers have been validated. CHD was defined as myocardial infarction, unstable angina pectoris, coronary revascularization (coronary artery bypass graft or percutaneous transluminal coronary angioplasty), or death due to CHD. CVD included CHD and ischemic stroke events. ACS was defined as MI, unstable angina or death due to CHD. The follow-up ended on Dec 31, 2010 in FINRISK and on Dec 31, 2008 in Health 2000.

Study protocols have been approved by the ethics committee of the National Institute for Health and Welfare, Finland, and/or the ethics committee of the Helsinki and Uusimaa Hospital District. All participants provided written informed consent.

SNP Selection and Genotyping

Out of 31 loci, which have been associated with myocardial infarction or coronary heart disease in genome-wide association studies, we included 28 SNPs in the study (Supplementary Table I). Three SNPs were excluded due to the failures in genotype assays or unreliable genotype calling. DNA samples were genotyped with the Sequenom MassARRAY System (Sequenom, San Diego, California), using iPLEX Gold chemistry and standard protocol. Genotyping was done at the Institute for Molecular Medicine Finland FIMM, and at the Wellcome Trust Sanger Institute, UK. All SNPs were in Hardy-Weinberg equilibrium and uncorrelated ($r^2<0.4$), and had the genotype call rate > 98% and sample call rate > 95%. We calculated the GRS as a weighted mean by using the reported effect sizes from the reference studies as weights for the risk allele counts, and divided the sum by the
number of the SNPs. Missing genotype data for each SNP was imputed with the average coded allele frequency of the study cohort.

**Statistical Methods**

CHD was the main cardiovascular end point in our analyses. We excluded individuals who were older than 75 years or had prevalent CVD at baseline. Participants reaching the age of 80 years during the follow-up were censored at their 80th birthday. Associations between the GRS and cardiovascular events were estimated with Cox proportional hazards models adjusted for the traditional risk factors at baseline: sex, total cholesterol, high-density lipoprotein (HDL) cholesterol, body mass index (BMI), systolic blood pressure, blood pressure treatment, current smoking status and diabetes mellitus. Age was used as the time scale in Cox models.

To further quantify the genetic effects for the subjects with different genetic risk load, we divided the GRS into deciles, and estimated the risk for each group by using the middle 20% of individuals as a reference. To estimate differences in risk between the subjects in the highest and middle values of the GRS, we compared the extreme 20%, 10% and 5% ends of the GRS with the middle 20% reference group. Deviation from the linear risk function was tested by fitting the joint model with both continuous GRS and an indicator variable, where the subjects in the highest 10% of the GRS were assigned as 1 and others as 0. We also studied the effects of unweighted GRS by calculating the number of risk alleles for each subject, and tested the association between cardiovascular end points and unweighted GRS using Cox models.

The information on family history of CVD was available in FINRISK 1992, FINRISK 1997 and FINRISK 2002 cohorts (N=19 001). We estimated the effect of family history on CHD, CVD and ACS using Cox models adjusted for traditional risk factors. We further adjusted the models for the GRS to examine how much the effects of these variants explain the familial risk. As a comparison, we studied the genetic effects with and without the adjustment for family history.

We found no evidence on heterogeneity in effect estimates between studies, and thus fixed effects meta-analysis was used to combine the results from each cohort. The validity of proportional hazards assumption was tested with scaled Schoenfeld residuals 12.

To evaluate the improvement in risk discrimination by using the genetic information and family history, we compared C-indices 13 for the models with and without the GRS and family history indicator in FINRISK cohorts. The change in C-index was estimated in each cohort separately and then combined across studies as proposed previously 14.

We then studied risk reclassification by using a restricted 14-year follow-up of FINRISK 1992 and 1997 cohorts. We modeled risk reclassification jointly in these two datasets and adjusted the analysis with the cohort indicator and traditional risk factors, including family history. Net reclassification improvement (NRI) was calculated from prospective data 15 using four risk categories: 0–5%, 5–10%, 10–20% and >20%. Clinical NRI was calculated for the subjects, who were classified to the intermediate risk group (10–20%) by the conventional model (model without the genetic data) 16. Since these risk categories are usually applied for 10-year
time period, we performed additional sensitivity analysis by rising all thresholds by 2%. We also calculated integrated discrimination improvement (IDI) \(^{17}\), explained relative risk \(^{16}\) and evaluated model calibration with Hosmer-Lemeshow goodness-of-fit test \(^{19}\).

Following the concept of two-stage risk screening used in two recent studies \(^{14,20}\), we estimated the clinical benefit of the GRS in a cardiovascular risk screening in a standard European population of 100 000 subjects. We assumed that all participants were first classified into cardiovascular risk categories based on traditional risk factors, and then additional GRS screening was targeted to those at the intermediate risk category (predicted risk 10–20%). The subjects at the intermediate risk were considered as clinically relevant subgroup based on the following assumptions; 1) statin medication is allocated to the subjects at the high risk category (≥20%) and the subjects with diabetes \(^{21,22}\), and 2) statins reduce cardiovascular risk by 20% in subjects without prevalent CVD \(^{23}\). Reclassification was calculated separately for males and females and in four age groups (40-50, 50-60, 60-70, ≥70). Assuming that age- and sex-specific incidences of CHD in the European standard population are comparable to the current study, we estimated incidence rates from the FINRISK 1992 and 1997. We weighed reclassification tables with estimated incidence rates multiplied by the group-specific counts based on standard European population.

The statistical package R (version 2.12.2) was used for all analyses. We considered two-sided P<0.05 to be statistically significant.

**REFERENCES**