RESEARCH LETTER

Genetic Polymorphisms for Estimating Risk of Atrial Fibrillation in the General Population: A Prospective Study

Atrial fibrillation (AF) is a common cardiac disease and major risk factor for stroke, heart failure, and death. Tools for prediction of AF have been developed to identify individuals who might benefit from preventive therapies, incorporating conventional cardiovascular risk factors, and the effects of such risk factors have been evaluated across several cohorts.1–3Recently, a heritable component to AF has been reported, and polymorphisms in 3 genetic regions have been reproducibly associated with AF: chromosome 4q25, located 150 kb from the closest gene—a transcription factor (PITX2) involved in cardiac development; chromosome 16q22, intrinsic to another transcription factor of unknown function, expressed in cardiac tissue (ZFHX3); and an amino acid–altering variant in KCNH2, one of the major cardiac voltage-gated potassium channels.1–3 Rare genetic variants segregating with AF are typically exclusive to individual families and unlikely to contribute to AF prediction at the population level, but genetic polymorphisms could provide important predictive information.

Methods. The single nucleotide polymorphism (SNP) with the strongest association at each of the 3 genetic regions reproducibly associated with AF in genome-wide4–6 or candidate gene studies7 was genotype in a large population-based cohort of middle-aged participants from southern Sweden (Malmo Diet and Cancer study8). Data collection and clinical definitions have been described previously.8 Briefly, 30,447 randomly selected individuals (born 1923–1950) attended a baseline examination between 1991 and 1996 with (1) sampling of venous blood, (2) measurement of blood pressure and anthropometric measures, and (3) completion of a questionnaire. Cardiovascular disease end points were ascertained from national registers (Swedish Cause of Death Register and Swedish Hospital Discharge Register). Follow-up for AF extended through January 1, 2009.

DNA extracted from peripheral blood cells was assigned to batches without regard to AF status or personal identity. The batches were genotyped with the same set of reagents using real-time polymerase chain reaction with 2.5 ng of DNA as the polymerase chain reaction template for allelic discrimination (ABI 7900HT; Life Technologies). Genotype calls were obtained using SDS version 2.3 software (Life Technologies) and fluorescence intensity plots curated manually.

Association of genotype with AF was studied using both cross-sectional and prospective study designs. In cross-sectional analyses, the association of SNPs with AF diagnosed before baseline was examined using logistic regression analysis. In prospective analyses, the association of SNPs with incident AF during follow-up was examined in individuals free of AF at baseline using Cox proportional hazards models with censoring at death, emigration, or end of follow-up. Kaplan-Meier estimates of absolute AF risk per genotype were calculated. The proportionality of hazards assumption was confirmed using a Schoenfeld global test.

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Polymorphisms associated with AF were assessed for predictive discrimination using the Harrell concordance (C) statistic, a generalization of the area under the receiver operating characteristic curve, with confidence interval estimates using a jackknife resampling method in the Stata package Somers D (Stata Corp). Model calibration was evaluated using the Grommesby-Borgan test implemented in the Stata package stcoxgof (Stata Corp). All analyses were performed using SAS version 9.2 (SAS Institute) or Stata version 11.1 (Stata Corp).

Informed consent was obtained from all participants, and the study was approved by the ethics committee of Lund University, Lund, Sweden. The study protocol is consistent with the principles of the Declaration of Helsinki.

Results. Baseline characteristics for the Malmo Diet and Cancer study cohort have been published previously.6 Clinical data were available for 28,473 individuals, 26,946 of whom had DNA available. The mean (SD) age was 58.1 (7.6) years, and the majority were women (60.6%). At baseline, 287 individuals had been diagnosed as having AF (prevalence, 1.0%). During a follow-up period of up to 17.8 years (median follow-up, 14.1 years; interquartile range, 12.9–15.7 years), 2050 individuals developed AF. The Kaplan-Meier estimate of cumulative AF incidence was 11.9% (95% CI, 10.7%–13.3%).

The call rate was higher than 95% for all 3 SNPs. Minor allele frequencies (MAFs) were similar to those in previous studies and the European panel of the HapMap project (4q25: T allele, MAF 10.1%; 16q22: A allele, MAF
The addition of genetic polymorphisms did not significantly improve prediction of AF independently of and with similar risk magnitude to single clinical risk factors. However, genetic polymorphisms did not significantly improve predictive accuracy when added to clinical risk factors. The findings do not support the utility of clinical genotyping for AF risk prediction with these SNPs, which are currently being marketed by commercial companies for direct-to-consumer genetic testing with provision of absolute genetic risk estimates.

The association with the K897T missense variant in KCNH2 was not replicated and also recently failed to replicate in a large case-control sample. These results do not support the large effect described in the initial report but cannot rule out a small effect.

### Table. Prediction of Atrial Fibrillation With Genetic Polymorphisms and Conventional Risk Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cross-sectional Results</th>
<th>Prospective Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>Age</td>
<td>2.12 (1.75-2.57)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.94 (1.48-2.54)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI</td>
<td>1.21 (1.03-1.42)</td>
<td>.07</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.91 (1.89-4.49)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>1.80 (1.13-2.87)</td>
<td>.02</td>
</tr>
<tr>
<td>History of MI</td>
<td>1.59 (0.95-2.67)</td>
<td>.04</td>
</tr>
<tr>
<td>History of HF</td>
<td>18.55 (9.86-34.91)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>rs2200733</td>
<td>2.15 (1.69-2.74)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>rs2106261</td>
<td>1.28 (1.02-1.61)</td>
<td>.03</td>
</tr>
<tr>
<td>KCNH2 (rs1805123)</td>
<td>0.86 (0.68-1.09)</td>
<td>.22</td>
</tr>
<tr>
<td>Age, sex, and conventional risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs22700733</td>
<td>0.751 (0.724-0.779)</td>
<td>11.7</td>
</tr>
<tr>
<td>rs2106261</td>
<td>0.740 (0.713-0.767)</td>
<td>9.5</td>
</tr>
<tr>
<td>rs22700733, rs2106261</td>
<td>0.751 (0.724-0.779)</td>
<td>5.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.755 (0.730-0.779)</td>
<td>4.1</td>
</tr>
<tr>
<td>BMI</td>
<td>0.745 (0.719-0.771)</td>
<td>6.0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.738 (0.711-0.765)</td>
<td>13.0</td>
</tr>
<tr>
<td>History of MI</td>
<td>0.743 (0.717-0.769)</td>
<td>16.6</td>
</tr>
<tr>
<td>History of HF</td>
<td>0.790 (0.724-0.777)</td>
<td>14.5</td>
</tr>
<tr>
<td>All conventional risk factors</td>
<td>0.776 (0.750-0.802)</td>
<td>2.1</td>
</tr>
<tr>
<td>Age, sex, conventional risk factors, and genetic polymorphisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs22700733</td>
<td>0.784 (0.757-0.812)</td>
<td>3.2</td>
</tr>
<tr>
<td>rs2106261</td>
<td>0.776 (0.749-0.804)</td>
<td>8.1</td>
</tr>
<tr>
<td>Both polymorphisms</td>
<td>0.785 (0.757-0.813)</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; HF, heart failure; HR, hazard ratio; MI, myocardial infarction; OR, odds ratio.

a The upper part of the table presents effect estimates with 95% CIs per risk factor from multivariable models, including conventional risk factors and genetic polymorphisms. Cross-sectional results refer to logistic regression models of prevalent cases at baseline, and prospective results refer to Cox proportional hazards models of incident cases during follow-up. Effect estimates for genetic polymorphisms are shown per risk allele for age per 10 years and for BMI (calculated as weight in kilograms divided by height in meters squared) per 5 U. P values refer to Wald χ² tests. The lower part of the table presents C statistics with 95% CIs and calibration statistics with corresponding P values for each model. Calibration refers to Hosmer-Lemeshow tests for cross-sectional analyses and Groennesby-Borgan likelihood ratio tests for prospective analyses.

17.4%; and KCNH2: G allele, MAF 21.2%). The 2 SNPs from genome-wide association studies were associated with both incident and prevalent AF, but the SNP in KCNH2 from candidate gene studies was not (Table). The Kaplan-Meier estimate of AF incidence was 27.7% (95% CI, 11.6%-57.4%) for homozygotes of the risk allele T of rs2200733 and 11.4% (95% CI, 9.9%-13.1%) for C allele homozygotes. For rs2106261, the Kaplan-Meier estimate of AF incidence was 18.4% (95% CI, 12.8%-26.0%) for homozygotes of the risk allele T and 11.6% (95% CI, 9.9%-13.5%) for C allele homozygotes. Few individuals were homozygous for risk alleles of both SNPs (n = 11), but these individuals had a high prevalence (9%, n = 1) and incidence (45%, n = 5) of AF.

Discrimination of AF with genotypes and conventional risk factors is shown in the Table. Age and sex at baseline showed high discrimination for prevalent (C, 0.737) and incident (C, 0.738) AF. Small but nonsignificant improvements in discrimination were observed with the addition of single conventional risk factors or genetic polymorphisms (all P > .05). The addition of all conventional risk factors to age and sex improved C statistics modestly for prevalent (C, 0.776) and incident (C, 0.750) AF. The addition of genetic polymorphisms further improved the C statistics for prevalent (C, 0.785) and incident (C, 0.755) AF, although these improvements were not significant (P = .73 and P = .39).

Comment. In this large, prospective study, 2 genetic polymorphisms with high prevalence in the population predicted AF independently of and with similar risk magnitude to single clinical risk factors. However, genetic polymorphisms did not significantly improve predictive accuracy when added to clinical risk factors. The findings do not support the utility of clinical genotyping for AF risk prediction with these SNPs, which are currently being marketed by commercial companies for direct-to-consumer genetic testing with provision of absolute genetic risk estimates.

The association with the K897T missense variant in KCNH2 was not replicated and also recently failed to replicate in a large case-control sample. These results do not support the large effect described in the initial report but cannot rule out a small effect.
Additional, independent SNPs on 4q25 have been associated with AF, and a polymorphism on chromosome 1q21 was recently associated with lone AF. Although these polymorphisms with smaller effects are unlikely to improve predictive accuracy, future studies will be needed to evaluate the predictive information content of genome-wide SNP data. Furthermore, recent studies have demonstrated that asymptomatic episodes of AF may not be uncommon and may confer increased stroke risk. Characterization of populations for such episodes might reveal that genotypic risks based on clinical AF are underestimates.

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Author Contributions: Dr Smith had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Smith, Newton-Cheh, Melander, and Platonov. Acquisition of data: Smith and Melander. Analysis and interpretation of data: Smith, Newton-Cheh, Almgren, Melander, and Platonov. Drafting of the manuscript: Smith and Melander. Critical revision of the manuscript for important intellectual content: Smith, Newton-Cheh, Almgren, Melander, and Platonov. Statistical analysis: Smith, Almgren, and Melander. Obtained funding: Smith, Melander, and Platonov. Administrative, technical, and material support: Melander and Platonov. Study supervision: Newton-Cheh, Melander, and Platonov.

Financial Disclosure: None reported.

Funding/Sponsorship: The Malmo Diet and Cancer study was made possible by grants from the Swedish Cancer Society, the Swedish Medical Research Council, the Swedish Dairy Association, the Albert Pählsson and Gunnar Nilsson Foundations, and the Malmö city council. Drs Smith, Melander, and Platonov gratefully acknowledge financial support from the Swedish Heart-Lung Foundation. Dr Newton-Cheh was supported by grants HL080025 and HL098283 from the National Institutes of Health, a Doris Duke Charitable Foundation Clinical Scientist Development Award, and a Burroughs Wellcome Fund Career Award for Medical Scientists. Dr Melander was supported by grants from the European Research Council (STG-282255), the Swedish Medical Research Council, the Medical Faculty of Lund University, the Skåne University Hospital in Malmö, the Albert Pählsson Research Foundation, the Crafoord Foundation, the Swedish National Health Service, the Hulda and Conrad Mossfelt Foundation, the Ernhold Lundströms Research Foundation, the King Gustaf V and Queen Victoria Fund, the Lennart Hanssons Memorial Fund, the Marianne and Marcus Wallenberg Foundation, and the Knut and Alice Wallenberg Foundation. Dr Platonov was supported by the Swedish National Health Service, Skåne University Hospital, and the Craaford Foundation.

Role of the Sponsors: The funding sources had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Additional Contributions: We thank all participants in the Malmo Diet and Cancer study for making this study possible. We also thank Marktëa Sjögen for technical support with genotyping.

INVITED COMMENTARY

Genetic Prediction for Common Diseases: Will Personal Genomics Ever Work?

A major promise of human genetics has been the use of genetic information to predict the risk of common diseases in order to prevent and treat these conditions more effectively. Most common diseases have a complex etiology, and genes are expected to explain much of their risk. However, even though PubMed already retrieves more than 2 million articles with "gene OR genetic" (n=2 015 109 as of February 10, 2011) and half (n=1 040 434) are tagged as "Human," there are formidable difficulties in materializing this promise.1

Genome-wide association studies have now successfully identified thousands of common genetic variants that influence the risk of complex diseases. Large-scale evidence, agnostic testing with stringent statistical criteria, and rigorous replication standards guarantee that this literature has high credibility. Nevertheless, the discovered gene variants do not markedly expand our predictive ability compared with what can be achieved by using only information from long-known traditional risk factors. In this issue of the Archives, Smith et al2 add another example for...