nature genetics

Common variants in *KCNN3* are associated with lone atrial fibrillation

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Atrial fibrillation (AF) is the most common sustained arrhythmia. Previous studies have identified several genetic loci associated with typical AF. We sought to identify common genetic variants underlying lone AF. This condition affects a subset of individuals without overt heart disease and with an increased heritability of AF. We report a meta-analysis of genome-wide association studies conducted using 1,335 individuals with lone AF (cases) and 12,844 unaffected individuals (referents). Cases were obtained from the German AF Network, Heart and Vascular Health Study, the Atherosclerosis Risk in Communities Study, the Cleveland Clinic and Massachusetts General Hospital. We identified an association on chromosome 1q21 to lone AF (rs13376333, adjusted odds ratio = 1.56; $P = 6.3 \times 10^{-12}$), and we replicated this association in two independent cohorts with lone AF (overall combined odds ratio = 1.52, 95% CI 1.40–1.64; $P = 1.83 \times 10^{-21}$). rs13376333 is intronic to *KCNN3*, which encodes a potassium channel protein involved in atrial repolarization.

AF is the most common arrhythmia and is responsible for a profound socioeconomic burden. Individuals with AF are at a fivefold increased risk of stroke, a fourfold increased risk of heart failure and a nearly twofold increased risk of dementia and death[1]. Known risk factors for typical AF include advanced age, male sex, obesity, hypertension, heart failure, valvular heart disease, hyperthyroidism and a family history of AF. A subset of cases with AF show no clear precipitant, have no overt structural heart disease, present at a young age and are referred to as having lone AF[1]. Individuals with lone AF are more likely to have symptomatic, paroxysmal episodes than older individuals with more typical forms of AF observed in the community. In many individuals with lone AF, discrete foci of ectopic electrical activity that initiate AF have been found to originate within the pulmonary veins. Although lone AF appears to have a more benign course than typical AF[2], many symptomatic individuals require treatment with an antiarrhythmic medication, electrical cardioversion, a catheter ablation procedure to electrically isolate the pulmonary veins, or a combination of therapies.

Familial aggregation and early onset are prominent features in subjects with lone AF, and nearly 30% of probands with lone AF have a first-degree relative with the disease[3]. Framingham Heart Study (FHS) investigators observed that the odds of developing AF were three times higher for individuals with at least one parent in whom AF was diagnosed before the age of 75 than in those without a parental history of AF[4]. Similarly, in a large study of Icelanders, the risk of developing AF was increased nearly fivefold if one parent was affected before the age of 60 (ref. 5).

A genome-wide association study (GWAS) in Icelanders identified a chromosome 4q25 locus that was associated with AF[6]. Within this locus, two noncoding SNPs were independently associated with AF. The SNP most strongly associated with AF, rs2200733, conferred a 1.71-fold increased odds of AF ($P = 6.1 \times 10^{-41}$), and rs10033464 caused a 1.42-fold increased odds of AF ($P = 3.1 \times 10^{-11}$)[6]. More recently, two independent GWAS identified an association on chromosome 16q22 to AF

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Received 22 October 2009; accepted 22 January 2010; published online 21 February 2010; doi:10.1038/ng.537

Table 1 Characteristics of the study populations

			Males		Age[a]		Age range[b]	Age of onset[c]		Age of onset range[d]	Hypertension		Body mass index[e]		Diabetes	
Study	Group	*n*	*n*	%	Mean	s.d		Mean	s.d.		*n*	%	Mean	s.d	*n*	%
AFNET	Cases	468	236	50.4	51.8	7.2	29–74	51.3	7.6	29–65	252	53.8	28.0	4.9	36	7.7
KORA S4	Controls	438	219	50.0	56.2	7.1	45–69	–	–	–	185	42.2	27.7	4.5	37	8.4
HVH	Cases	163	97	59.2	58.5	6.9	36–68	55.7	7.0	33–65	83	50.6	32.6	8.4	18	11.0
CHS	Controls	1,773	625	35.3	69.3	2.6	65–74	–	–	–	839	47.3	26.5	4.5	194	10.9
ARIC	Cases	146	79	54.1	52.9	5.4	45–64	60.5	3.5	48–65	67	45.9	28.2	5.7	12	8.2
	Controls	9,361	4,185	44.7	53.9	5.6	44–64	–	–	–	2,153	23.0	26.7	4.7	706	7.5
Cleveland Clinic	Cases	183	147	80.0	52.4	9.3	20–65	45.5	10.9	16–64	95	51.6	30.9	6.4	24	13.0
	Controls	164	55	34.0	60.4	7.2	50–88	–	–	–	91	55.5	30.5	6.8	29	17.7
MGH[a]	Cases	375	304	81.1	53.4	10.6	21–77	46.1	11.7	13–65	85	22.6	27.8	5.0	12	3.2
FHS	Controls	1,108	500	45.1	59.4	9.8	20–74	–	–	–	438	39.6	27.9	5.3	78	7.0

[a]Age at blood draw in years; mean ± s.d. [b]Age range of study participants at blood draw. [c]Age of onset of AF in years; mean ± s.d. [d]Range of age of onset of AF in years. [e]Measured in kg/m^2. MGH, Massachusetts General Hospital Atrial Fibrillation Study; FHS, Framingham Heart Study; CHS, Cardiovascular Health Study.

cases typically observed in the community[7,8]. rs2106261 within *ZFHX3* was associated to AF with a risk ratio of 1.25 ($P = 1.8 \times 10^{-15}$)[7].

Using well-defined and carefully phenotyped subsets of individuals with a certain disease can facilitate gene discovery for complex traits, such as AF. Lone AF remains a diagnosis of exclusion (whereby a diagnosis is made by elimination of other reasonable conditions) and a consensus definition has yet to emerge[9]. In the current study, lone AF was defined as AF with an onset before 66 years of age and without a preceding history of a myocardial infarction, heart failure or known left ventricular systolic dysfunction. Because individuals with lone AF show a high heritability of AF and a distinct clinical presentation, we sought to identify the common genetic factors underlying lone AF.

Genome-wide genotyping was available for a total of 1,335 individuals with lone AF and 12,844 referent individuals from five centers (**Table 1** and Online Methods). After application of the SNP exclusion criteria in each study (**Supplementary Table 1**), the genomic inflation factor (λ) ranged from 0.98 in Atherosclerosis Risk in Communities Study (ARIC) to 1.17 in the Heart and Vascular Health (HVH) and Cardiovascular Health Studies (CHS), and the overall genome-wide meta-analysis λ was 1.017. We generated a quantile-quantile plot of the expected versus observed *P* value distributions for associations of the approximately 2.5 million SNPs from individuals with lone AF (**Supplementary Fig. 1a**). We also plotted the results of the lone AF meta-analysis with the $-\log_{10}$(*P* value) against physical coordinates on 22 autosomal chromosomes (**Fig. 1**). The most significant association with lone AF was at the previously reported 4q25 locus, with 77 SNPs that exceeded our genome-wide significance threshold of $P = 5 \times 10^{-8}$. The most significant SNP at this locus was rs6843082 (OR 2.03, 95% CI 1.79–2.30; $P = 2.5 \times 10^{-28}$; **Table 2**, **Fig. 1**, **Supplementary Fig. 2a** and **Supplementary Table 2**). Given the large number of significant associations at this locus, we produced a modified quantile-quantile plot without the 4q25 SNPS (**Supplementary Fig. 1b**).

A second locus identified on chromosome 1q21 had 6 SNPs that exceeded genome-wide significance. The most significant SNP was rs13376333, with an OR of 1.56 (95% CI 1.38–1.77, $P = 6.3 \times 10^{-12}$; **Table 2**, **Figs. 1** and **2** and **Supplementary Table 2**). rs13376333 is located in the intron between the first and second exon of the calcium-activated potassium channel gene *KCNN3*. We created a regional plot of the locus on chromosome 1q21 (**Fig. 2**). Our findings at the chromosome 1q21 locus were replicated in two studies of lone AF. First, in 977 cases from the German Competence Network on Atrial Fibrillation (AFNET) and 3,042 controls without AF from Cooperative Health Research in the Region of Augsburg (KORA) S4, rs13376333 was significantly associated with lone AF (OR 1.45, 95% CI 1.26–1.66, $P = 8.8 \times 10^{-8}$). Second, the association was replicated in the Vanderbilt University Lone AF Registry consisting of 187 subjects with lone AF and 565 control subjects without AF (OR 1.55, 95% CI 1.19–2.03, $P = 0.001$). In a meta-analysis combining the lone AF results from the primary GWAS and the two replication cohorts, rs13376333 had an OR of 1.52 (95% CI 1.40–1.64, $P = 1.83 \times 10^{-21}$).

There was no evidence of an interaction between rs6843082 and rs13376333, the two most significant SNPs at the loci on chromosomes 4q25 and 1q21, respectively ($P = 0.46$). We also sought to determine whether rs13376333 was associated with more typical forms of AF observed in the community. Using GWAS data from two independent studies in Rotterdam and Reykjavik (Age, Gene/Environment Susceptibility Study or AGES), we found that rs13376333 was associated with typical AF, albeit with a weaker effect (**Supplementary Table 3**)[7]. In a meta-analysis of the associations between this SNP and both incident and prevalent AF in Rotterdam and AGES, rs13376333 had an OR of 1.13 (95% CI 1.04–1.24, $P = 0.006$).

A third SNP, rs13038095 on chromosome 20q13, also exceeded genome-wide significance (OR 1.61, 95% CI 1.37–1.91, $P = 1.1 \times 10^{-8}$; **Table 2** and **Supplementary Table 2**) and is located upstream from *SULF2*, which encodes endosulfatase (**Supplementary Fig. 2b**). However, in the AFNET and Vanderbilt studies, the association of this SNP with AF was in the opposite direction and thus it failed to replicate (**Table 3**). Finally, we confirmed the recent locus for AF on chromosome 16q22 described in a meta-analysis of GWAS data from five longitudinal cohorts with more typical forms of AF observed in the community. The minor allele of rs2106261 was associated with lone AF with an odds ratio of 1.47 (95% CI 1.39–1.54, $P = 1.61 \times 10^{-7}$).

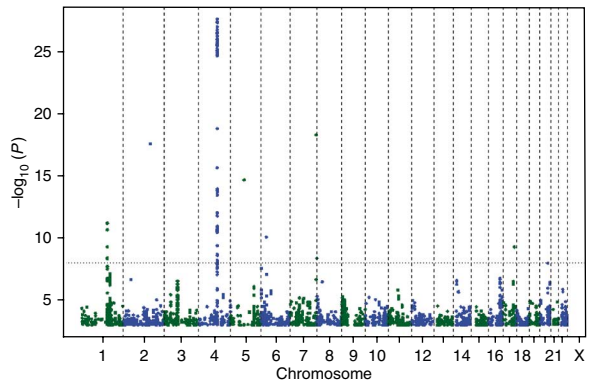

Figure 1 Manhattan plot of meta-analysis results for genome-wide association to lone AF. The $-\log_{10}$(*P* value) is plotted against the physical positions of each SNP on each chromosome. The threshold for genome-wide significance, $P < 5 \times 10^{-8}$, is indicated by the dashed line.

Table 2 Summary of GWAS meta-analysis results with $P < 5 \times 10^{-8}$

Locus				Cohort specific					Meta-analysis association signal						
SNP	Chr.	Position[a]	Closest gene	AFNET & KORA S4[b]	HVH & CHS[b]	ARIC[b]	CCF[b]	MGH & FHS[b]	Minor/major allele	MAF (%)	Meta-analysis[a]	OR	95% CI	Meta *P* value	Corroborating SNPs[c]
rs6843082	4	111,937,516	*PITX2*	0.77 (0.13)	0.43 (0.13)	0.55 (0.15)	0.87 (0.19)	0.91 (0.12)	G/A	25.8	0.71 (0.06)	2.03	1.79–2.30	2.5×10^{-28}	77
rs13376333	1	153,080,977	*KCNN3*	0.30 (0.13)	0.54 (0.13)	0.32 (0.14)	0.66 (0.20)	0.52 (0.12)	T/C	29.5	0.45 (0.06)	1.56	1.38–1.77	6.3×10^{-12}	6
rs13038095	20	45,858,983	*SULF2*	0.28 (0.20)	0.45 (0.10)	0.61 (0.19)	0.85 (0.29)	N/A[d]	T/G	10.2	0.48 (0.08)	1.61	1.37–1.91	1.1×10^{-8}	0

[a]Genomic position from NCBI Build 36. [b]β (s.e.m.). [c]Having $P < 5 \times 10^{-8}$. [d]MGH/FHS did not contribute to the meta-analysis of this SNP due to poor quality imputation. Chr., chromosome; MAF, minor allele frequency; OR, odds ratio, CI, confidence interval; N/A, not available. β is the regression parameter estimate (the log-odds ratio) and OR is the odds ratio of lone AF for each additional minor allele.

Variants at the 1q21 locus cluster at *KCNN3* (also known as *SK3* and *KCa2.3*), which codes for a member of a family of voltage-independent calcium-activated potassium channels. These small conductance channels are expressed in a number of excitable tissues including the brain[10] and heart[11,12]. In neurons, these channels underlie the classical afterhyperpolarization or inward potassium current that is activated by elevations in calcium following repetitive action-potential stimulation. The role of these channels in the heart is less clear. In a rabbit burst-pacing model designed to simulate pulmonary venous ectopy, pharmacologic blockade of KCNN channels inhibited pacing-induced shortening of pulmonary venous and atrial action potential duration[13]. Shortening of the atrial action potential duration reduces the refractory period of atrial myocytes and promotes re-entry, an important mechanism for the development and maintenance of AF[14]. Although no change was observed in *KCNN3* expression in response to burst pacing[13], expression of a closely related family member, *KCNN2*, was increased. Differential expression of Kcnn2 in a mouse model is associated with altered pacemaker-cell action-potential duration and spontaneous firing rate[15]. KCNN3 and KCNN2 co-assemble in heteromultimeric channel complexes *in vitro*[16]; therefore, genetic variation in either subunit may modulate channel function. However, we did not identify any SNPs within 60 kb of *KCNN2* that were significantly associated with lone AF (defined as $P < 0.001$, data not shown). The KCNN channels also are expressed in vascular endothelial cells, and suppression of *Kcnn3* expression in a mouse model has been associated with increased blood pressure[17]. The most significant SNP at this locus, rs13376333, is not in linkage disequilibrium (LD) with any known common, nonsynonymous SNP in *KCNN3*[18]. Thus, the mechanism by which variation at this locus increases AF risk is unknown. Although *KCNN3* is a plausible candidate gene for lone AF, additional studies on the role of this channel and other genes at this locus are warranted. Future work directed at determining the role of the SNPs identified for lone AF in predicting the progression from paroxysmal to permanent AF, or in defining subtypes of lone AF, will be of interest. Furthermore, the utility of *KCNN3* as a potential direct or indirect target for the pharmacological treatment of AF may also merit further investigation.

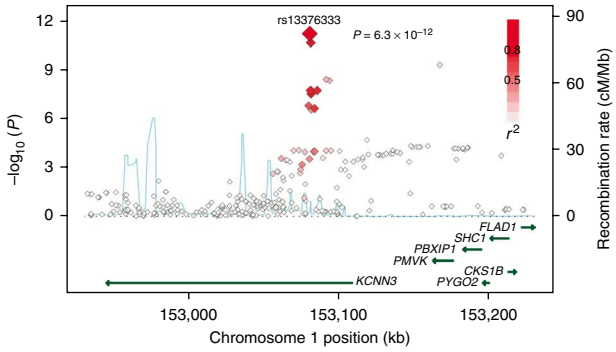

Figure 2 Regional plot for locus on chromosome 1 associated with lone atrial fibrillation. Figures were prepared using SNAP[20]. SNPs are plotted with the meta-analysis *P* value and genomic position (NCBI Build 36). The SNP of interest is labeled. The strength of the LD is indicated by gradient of red. Blue line indicates estimated recombination rates and dark green arrows indicate gene annotations. LD and recombination rates are based on the CEU HapMap release 22.

To minimize false positive associations that may arise from GWAS, we used a conservative threshold for genome-wide significance and independently replicated our findings in additional subjects with lone AF. To avoid the confounding influence of population stratification, our analyses were restricted to subjects of European ancestry, so the generalizability of our findings to individuals of non-European ancestry may be limited. Additionally, we included subjects with hypertension in our analysis both because of the high lifetime risk of hypertension and also to maximize the power of our study. However, it is unlikely that hypertension confounded our results because rs13376333 was not associated with systolic or diastolic blood pressure in the Global BPgen Consortium, a meta-analysis of genome-wide association data for blood pressure in over 34,000 individuals from 13 cohorts of European descent (systolic blood pressure +0.2 mm Hg per minor allele, 95% CI −0.1 to +0.5, $P = 0.27$ and diastolic blood pressure +0.1 mm Hg per minor allele, 95% CI −0.1 to +0.3, $P = 0.25$)[19]. Further, our results were replicated in individuals from the Vanderbilt study with lone AF in the absence of hypertension. Although we were not able to perform a multivariate analysis adjusting for other associated risk factors for AF, rs1337633 was not associated with body mass index or left atrial size, two other common risk factors for AF (data not shown). Because lone AF is a diagnosis of exclusion, there were variations in the enrollment procedures between sites. In particular, an echocardiogram was not performed on subjects in the ARIC study or on approximately 30% of the subjects in the HVH study. Nevertheless, the identified association at *KCNN3* remained consistently replicated in independent populations with lone AF. Finally, the associations we observed do not necessarily imply that the candidate genes discussed are directly involved in the pathogenesis of AF. The associated SNPs may lie within or serve as proxies for noncoding regulatory elements that may affect gene expression at considerable distances from their respective genomic locations.

In summary, we identified a new locus for lone AF at the calcium activated potassium channel gene, *KCNN3*. Future studies will seek to determine the mechanistic links between genetic variation at this locus and AF.

Table 3 Replication of the association between SNPs on chromosomes 1q21 and 20q13 with atrial fibrillation in cohorts with lone atrial fibrillation

			rs13376333 (Chr. 1q21)				rs13038095 (Chr. 20q13)			
Study	AF[a]	No AF[a]	MAF[b]	OR	95% CI	*P* value	MAF[b]	OR	95% CI	*P* value
AFNet & KORAS4[c]	977	3,042	35.7/29.0	1.45	1.26–1.66	8.8×10^{-8}	9.9/10.5	0.81	0.65–1.01	0.053
Vanderbilt Lone AF Registry[d]	186	565	39.5/32.8	1.55	1.18–2.04	0.0012	8.5/9.1	0.89	0.57–1.40	0.630

[a]*n*, total number of participants in each category. [b]MAF, minor allele frequency for the cases/controls. [c]Adjusted for age, sex and hypertension. [d]Adjusted for age and sex. OR, odds ratio for lone AF with each additional copy of the minor allele; CI, confidence interval.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Accession codes. *KCNN3* is deposited in GenBank with the accession number NM_002249.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

Acknowledgements are contained in the **Supplementary Note**.

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Obtained funding: P.T.E., A.P., A.A., M.K.C., M.F.S., P.I.W.d.B., M.M., S.A.L., E.F., N.L.S., J.D.S., K.M.R., D.R.V.W., J.I.R., S.L.H., S.M., B.H.Ch.S., A.H., A.G.U., D.L., E.B., A.M., E.J.T., A.C., V.G., B.M.P., D.M.R., T.M., J.C.M.W., J.B., D.E.A., E.J.B., S.R.H. and S.K.
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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

Subjects and definitions. Lone AF was defined as AF with an onset before 66 years of age and without a preceding history of a myocardial infarction, heart failure or known left ventricular systolic dysfunction. Referent subjects in all studies were free from AF as documented by electrocardiography. In each center, the local institutional review boards reviewed and approved all study procedures; written, informed consent was obtained from each study subject. All subjects were of European descent. None of the cases or controls were related. None of the GWAS data in the individuals with lone AF from MGH, AFNET, HVH or CC have previously been reported. Cases with lone AF from the ARIC study were included in a meta-analysis of the genetic determinants of AF observed in the community[7].

Description of study cohorts. AFNET[21] is a national registry of AF patients. In this registry, DNA samples have been collected from patients with onset of AF before the age of 60 at the Medical Department I of the University Hospital Munich, Campus Grosshadern, of the Ludwig-Maximilians Universität Munich. Cases were selected if the diagnosis of AF was made on an electrocardiogram that was analyzed by a trained physician. Patients with signs of moderate to severe heart failure or moderate to severe valve disease or with hyperthyroidism were excluded from the study. Referent subjects were drawn from the KORA S4 study[22], with ages ranging from 25 to 74 years, had no history of AF, myocardial infarction, heart failure or valve disease, and had documented sinus rhythm at the time of blood draw.

The Massachusetts General Hospital Atrial Fibrillation Study (MGH)[3] enrolled serial patients with lone AF or AF and hypertension who were referred to the arrhythmia service between 5 July 2001 and 19 February 2008. Inclusion criteria were AF that had been documented by electrocardiography and age less than 66 years. Individuals with structural heart disease as assessed by echocardiography, hyperthyroidism, myocardial infarction or heart failure were excluded. All patients were evaluated by 12-lead electrocardiogram, echocardiogram and laboratory studies. Referent subjects were selected from FHS[23–25], and included unrelated subjects 18–74 years of age with no history of AF at blood draw or in follow-up and with no history of myocardial infarction, heart failure or valve disease at baseline.

HVH[26] classified all enrollees of Group Health, a large health plan in Washington state, with a new International Classification of Diseases, 9th revision (ICD-9) code for AF between 1 October 2001 and 31 December 2004. Incident AF was verified by review of medical records with the requirement that the AF be documented by 12-lead electrocardiogram and clinically recognized by a physician, with no previous evidence of AF in the medical record. Cases with early-onset AF were those less than 66 years of age at diagnosis, without a history of coronary artery disease, valvular disease, heart failure, poor left ventricular function, chronic obstructive pulmonary disease, active cancer or hyperthyroidism. Referent subjects were identified from the CHS[27] and were 65–74 years old, had no history of AF at blood draw or in follow-up, and had no history of myocardial infarction, heart failure or valve disease at baseline.

Cases of early-onset AF were identified from the ARIC Study[28]. Study participants underwent electrocardiograms at baseline and at each follow-up exam (3 exams; one exam every 3 years). As part of standard follow-up procedures, trained abstractors obtained and recorded all ICD-9 hospital discharge diagnosis information from participants' hospitalizations reported in annual follow-up interviews. AF was defined as the presence of ICD-9 code 427.31 in the discharge codes. Also, ARIC participants were classified as AF cases if their underlying cause of death was AF (ICD-10 code I48 or ICD-9 code 427.3). In this analysis, cases of early-onset AF were identified by any of the three mentioned sources (scheduled study electrocardiogram, hospital discharge ICD code or death certificate) in the absence of any clinical evidence of structural heart disease. Referent subjects in the ARIC study were 45–64 years of age, had no history of AF at the baseline or in follow-up and had no history of a myocardial infarction, heart failure or valvular heart disease at baseline; only subjects of European descent were used for analysis.

The Cleveland Clinic Lone AF GeneBank Study consists of subjects from the Cleveland Clinic Lone Atrial Fibrillation GeneBank. Subjects included in this analysis were at least 18 years of age and less than 66 years of age, with a history of recurring or persistent lone AF, ≤50% coronary artery stenosis in the coronary arteries or with normal stress test results, and had normal left ventricular ejection fraction ≥50%. Subjects were excluded if they had a history of severe valvular disease or coronary artery disease, or a prior myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft; congenital heart disease, hypertrophic cardiomyopathy, aortic dissection or repair; or latest left ventricular ejection fraction < 50%. Referent subjects were drawn from the GeneBank at the Cleveland Clinic Heart Center and had been genotyped as part of a prior case-control GWAS of myocardial infarction. Referent subjects were included if they had no history of AF at any age and otherwise met the inclusion criteria as described above for the early-onset AF case cohort.

Replication cohorts. Unique early-onset AF cases from AFNET that were not genotyped in the initial GWAS were used as a replication cohort. The Vanderbilt Lone AF Registry[29] consists of patients between 18 and 65 years of age with documented AF in the absence of hypertension, heart failure, coronary disease, or substantial valve disease by echocardiography. Consecutive patients with AF were prospectively enrolled beginning in October 2002 from the Vanderbilt Cardiology and Arrhythmia Clinics, the emergency department and inpatient services[29]. Referent subjects were free from AF as documented by electrocardiography.

Genotyping. For lone AF, the genotyping platform used in each study included: Affymetrix 6.0 (MGH and ARIC), Affymetrix 5.0 (FHS), Illumina 370 CNV (HVH and CHS), Illumina 330 (AFNET and KORA S4), Illumina Hap550 v3 and Illumina Hap610 v1 (Cleveland Clinic Cases), and Perlegen GV4 (Cleveland Clinic Controls). Additional information on the genotyping exclusions in each cohort are provided in **Supplementary Table 1**. Replication genotyping was performed for rs13376333 and rs13038095 using a TaqMan assay (Applied Biosystems, Inc.) in the Vanderbilt Lone AF Registry, and an iPlex single base primer extension with MALDI-TOF mass spectrometry (Sequenom) was used for AFNET and KORA S4. The AGES and Rotterdam Studies were genotyped on the Illumina CNV370 and Infinium HumanHap550 platforms, respectively.

Statistical analysis. For the lone AF meta-analysis, over 2.5 million HapMap SNPs were imputed within each study using the CEU population. Mach v1.0.1x (see URLs) was used for analysis of AFNET, KORA S4, MGH, FHS, ARIC and Cleveland Clinic; BIMBAM[30] was used for HVH and CHS data. In studies for which population structure was associated with the lone-AF phenotype (FHS and MGH, CHS and HVH, and Cleveland Clinic), analyses were adjusted for the principal components of genotype associated with phenotype[31] or were stratified by identity-by-descent cluster group using the method implemented in PLINK[32]. The primary GWAS analysis in each center used logistic regression, adjusting for age at DNA draw, sex and hypertension status. ARIC also adjusted for study site. The HVH and CHS analysis was not adjusted for age because of limited overlap in ages between cases and controls. Each SNP was modeled using an additive genetic effect. The ratio of observed to expected variance in the imputed SNP genotype counts was used as a quality control metric for imputed SNPs[33]. For each SNP, all studies with variance ratios greater than 0.10 were included in meta-analyses. A fixed-effects model was used for meta-analysis of the genotype logistic regression parameters (log odds ratios), using inverse variance weights, as implemented in the meta-analysis utility METAL (see URLs). Prior to meta-analysis, genomic control was applied to each study with a genomic control inflation factor (λ) >1.0 by multiplying the standard error of the SNP regression parameter by the square-root of the study-specific λ. A total of 2,445,595 SNPs with average MAFs greater than or equal to 0.01 across participating studies were included in meta-analyses. To guard against reporting false positives due to data from a single study, we omitted SNPs with available results from fewer than three studies and SNPs with extreme results in a single study ($P < 10^{-8}$) but with no consistent support at $P < 0.05$ from other studies. We prespecified a $P < 5 \times 10^{-8}$ corresponding to Bonferroni adjustment for 1 million independent tests[18], as our criterion for genome-wide significance.

We sought to determine whether the top SNP at each locus for lone AF that exceeded our threshold for genome-wide significance was also associated with more typical forms of AF observed in the community by performing an *in silico* replication in the AGES and the Rotterdam Study.

URLs. Mach v1.0.1x, http://www.sph.umich.edu/csg/abecasis/MACH/; METAL, http://www.sph.umich.edu/csg/abecasis/metal/index.html.

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