Meta-analysis identifies six new susceptibility loci for atrial fibrillation

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Abstract

Atrial fibrillation is a highly prevalent arrhythmia and a major risk factor for stroke, heart failure and death[1]. We conducted a genome-wide association study (GWAS) in individuals of European ancestry, including 6,707 with and 52,426 without atrial fibrillation. Six new atrial fibrillation susceptibility loci were identified and replicated in an additional sample of individuals of European ancestry, including 5,381 subjects with and 1 0,030 subjects without atrial fibrillation ($P < 5 \times 10^{-8}$). Four of the loci identified in Europeans were further replicated *in silico* in a GWAS of Japanese individuals, including 843 individuals with and 3,350 individuals without atrial fibrillation. The identified loci implicate candidate genes that encode transcription factors related to cardiopulmonary development, cardiac-expressed ion channels and cell signaling molecules.

Genome-wide association studies in individuals of European descent have identified three genomic regions associated with atrial fibrillation on chromosomes 4q25 (*PITX2*)[2–5], 16q22 (*ZFHX3*)[3,5] and 1q21 (*KCNN3*)[2]. Studies of electrocardiographic traits have also identified a number of loci associated with atrial fibrillation[6,7]. However, despite these findings, much of the heritability of atrial fibrillation remains unexplained, justifying the search for additional genetic variants underlying atrial fibrillation risk[8]. Large-scale meta-analysis of GWAS results is a powerful method to identify additional genetic variation underlying traits

and conditions. We therefore conducted a meta-analysis of multiple well-phenotyped GWAS samples of European ancestry to identify additional atrial fibrillation susceptibility loci.

Six prospective cohort and 10 prevalent study samples contributed to the discovery analysis, which was adjusted for age and sex (Table 1, Online Methods and Supplementary Note). Atrial fibrillation status was systematically ascertained in each sample (Online Methods and Supplementary Note). After application of quality control SNP exclusion criteria in each study (Supplementary Table 1), meta-analysis was performed, applying genomic control to each study. The genomic control inflation factor for the meta-analysis was 1.042 for the full set of SNPs and 1.040 after omitting all SNPs within 500 kb of the association signals that reached genome-wide significance. The quantile-quantile plot of the expected versus observed P-value distributions for association of the 2,609,549 SNPs analyzed is shown (Supplementary Fig. 1). We identified ten loci that exceeded our preset threshold for genome-wide significance ($P < 5 \times 10^{-8}$) (Fig. 1). The three loci most significantly associated with atrial fibrillation were at previously identified atrial fibrillation susceptibility loci on chromosomes 4q25 in *PITX2* (rs6817105; $P = 1.8 \times 10^{-74}$)[4], 16q22 in *ZFHX3* (rs2106261; $P = 3.2 \times 10^{-16}$)[3,5] and 1q21 in *KCNN3* (rs6666258; $P = 2.0 \times 10^{-14}$)[2] (Table 2).

Seven new genomic loci were associated with atrial fibrillation with $P < 5 \times 10^{-8}$ in the discovery stage (Table 2). The most significantly associated SNP in each of the seven new loci was genotyped and tested for association with atrial fibrillation in an additional 3,132 to 5,289 independent individuals with atrial fibrillation and 8,159 to 11,148 referent individuals derived from six studies of individuals of European ancestry (Supplementary Table 2). Six of the loci associated with atrial fibrillation in the discovery stage met our criteria for independent replication. Study-specific replication results are detailed (Supplementary Table 3). The results from meta-analysis of the discovery and replication results are shown (Table 2), as are regional plots (Fig. 2). Recognizing that the genes in closest physical proximity to the associated SNPs are not always the causative genes, we report below the genetic associations in order of statistical significance along with the nearest gene.

The most significant new association in the discovery stage was on chromosome 1q24 (rs3903239; overall $P = 8.4 \times 10^{-14}$) in *PRRX1*, which encodes a homeodomain transcription factor highly expressed in the developing heart, particularly in connective tissue. Biological interaction between *PRRX1* and a related homeobox transcription factor gene, *PRRX2*, results in abnormalities of great vessel development in a mouse knockout model[9]. In a separate *PRRX1* knockout, fetal pulmonary vasculature development was impaired[10].

A second locus was identified on chromosome 7q31 (rs3807989; overall $P = 3.6 \times 10^{-12}$) in *CAV1*, which encodes caveolin-1, a cellular membrane protein involved in signal transduction. *CAV1* is selectively expressed in the atria[11], and its knockout has been associated with dilated cardiomyopathy[12]. The CAV1 protein colocalizes with and negatively regulates the activity of KCNH2 (ref. 13), a potassium channel involved in cardiac repolarization; the corresponding *KCNH2* gene was found to be associated with atrial fibrillation in a candidate gene association study, although not in our present analysis[14]. The top SNP at the *CAV1* locus identified in the current study, rs3807989, was previously identified in a GWAS of the PR and QRS intervals and related to atrial fibrillation[6,7]. The relationship between other previously reported PR-associated loci and atrial fibrillation are reported (Supplementary Table 4). Of note, significant associations

with atrial fibrillation were observed for SNPs related to the PR interval in *SOX5*, *TBX5*, *SCN5A* and *SCN10A*[6,7].

The third locus on chromosome 14q23 (rs1152591; overall $P = 5.8 \times 10^{-13}$) was located in an intron of *SYNE2*, which encodes numerous nesprin-2 isoforms, some of which are highly expressed in the heart and skeletal muscle. Nesprin-2 localizes throughout the sarcomere and is involved in maintaining nuclear structural integrity by anchoring the nucleus to the cytoskeleton. In a candidate gene approach, mutations in *SYNE2* were found to segregate in some families with Emery-Dreifuss muscular dystrophy[15], which is characterized by skeletal muscle atrophy, cardiomyopathy and cardiac conduction defects.

The fourth locus on chromosome 9q22 (rs10821415; overall $P = 4.2 \times 10^{11}$) was located in an ORF on chromosome 9. Genes at this locus include *FBP1* and *FBP2*, which are important for gluconeogenesis. Autosomal recessive *FBP1* deficiency has been described, but cardiovascular features did not seem to be prominent[16]. Variants at 9q22 have been implicated in the regulation of height, pulmonary function, angiogenesis and attention deficit hyperactivity disorder, although rs10821415 is not in substantial linkage disequilibrium (LD) with any of these SNPs ($r^2 < 0.30$).

A fifth signal was located at 15q24 (rs7164883; overall $P = 2.8 \times 10^{-17}$) in the first intron of *HCN4*. The HCN4 protein is the predominant cardiac hyperpolarization-activated cyclic nucleotide–gated channel and is highly expressed in the sinoatrial node. HCN4 activity underlies the funny current (I_f) that governs cardiac pacemaking, and mutations in *HCN4* have been associated with various forms of sinus nodal dysfunction[17,18].

A sixth locus on chromosome 10q22 (rs10824026; overall $P = 4.0 \times 10^{-9}$) was located 5 kb upstream of *SYNPO2L* and 20 kb upstream of *MYOZ1*. The proteins encoded by *SYNPO2L* and *MYOZ1* are both expressed in skeletal and cardiac muscle, localize to the Z-disc and interact with numerous other proteins. However, the precise role of either gene in cardiovascular physiology is unknown[19,20]. A mouse knockout of *MYOZ1* showed increased calcineurin activity and cardiac hypertrophy in response to pressure overload. However, candidate gene approaches have not supported a prominent role for *MYOZ1* mutations in causing familial dilated cardiomyopathy[21]. Of note, the *SYNPO2L* locus is located within a previously reported atrial fibrillation susceptibility locus identified in a family with autosomal dominant atrial fibrillation[22].

One other locus was identified in the meta-analysis of atrial fibrillation in the *WNT8A* gene (rs2040862; $P = 3.2 \times 10^{-8}$); however, this association failed to replicate in additional independent cohorts with atrial fibrillation (replication $P = 0.36$; combined $P = 2.5 \times 10^{-7}$).

There was evidence of significant heterogeneity in the discovery meta-analysis at the previously published atrial fibrillation susceptibility signals at 4q25 in *PITX2* and at 16q22 in *ZFHX3* (Table 2). Effect heterogeneity at the *PITX2* locus has already been observed[4,23,24].

We then sought to determine whether the top SNPs or their proxies at each locus were associated with alterations in gene expression in an expression quantitative trait locus (eQTL) database, the Genotype-Tissue Expression eQTL browser. The top SNP at the *SYNPO2L* locus, rs10824026, is in strong LD with a SNP, rs12570126 ($R^2 = 0.932$), that was found to correlate with the expression of *MYOZ1* and *SYNPO2L* ($P = 1.5 \times 10^{-6}$; data derived from lymphoblastoid cell lines in 270 individuals from the HapMap Consortium)[25]. Furthermore, the top SNP at the *SYNPO2L* locus is in LD with a nonsynonymous SNP in *SYNPO2L*, rs3812629 ($R^2 = 0.8$; encoding the SYNPO2L P707L variant with respect to transcript Q9H987-1), that is predicted to be damaging by both the PolyPhen-2 and the SIFT

algorithms. None of the other identified atrial fibrillation risk SNPs were associated with variations in gene expression in the Genotype-Tissue Expression eQTL browser.

We next examined the generalizability of our findings by examining our results in a separate GWAS of individuals of Japanese ancestry, including 843 subjects with and 3,350 subjects without atrial fibrillation in the Japan BioBank study (Supplementary Fig. 2a,b). Within the Japanese GWAS, only the chromosome 4q25 (*PITX2*) locus exceeded the preset threshold for genome-wide significance (rs2634073; odds ratio (OR) = 1.84, 95% confidence interval (CI) = 1.59–2.13; 3.7×10^{-17}; Supplementary Figs. 2a and 3 and Supplementary Table 5). At the previously published locus at 16q22 (*ZFHX3*)[3,5], rs12932445 was associated with atrial fibrillation in participants of Japanese ancestry ($P = 6.8 \times 10^{-4}$). The relationship between atrial fibrillation and variants at the *KCNN3-PMVK* locus on chromosome 1q21 failed to replicate ($P = 0.17$); however, a regional plot of this locus revealed a distinct signal at the rs7514452 SNP—approximately 375 kb away from rs6666258, the top SNP in the European ancestry sample—that was modestly associated with atrial fibrillation in the Japanese sample ($P = 4.9 \times 10^{-5}$; $R^2 = 0.002$). At *PRRX1* and *CAV1*, the top SNPs in the European samples were also associated with atrial fibrillation in Japanese individuals. For the loci near *PRRX1* and *C9orf3,* alternate SNPs in the Japanese cohort were more significantly associated with atrial fibrillation than the top SNP at each locus in Europeans (Supplementary Fig. 3 and Supplementary Table 5).

Our study was subject to a number of limitations. To maximize both the power and the generalizability of our study, we included all available individuals with atrial fibrillation; thus, some individuals had comorbidities, such as systolic dysfunction and hypertension. However, none of the identified risk variants for atrial fibrillation were strongly associated with systolic dysfunction in the EchoGen Consortium[26], a meta-analysis of echocardiographic data from 5 community-based cohorts consisting of over 12,000 individuals of European descent (P value $< 1 \times 10^{-5}$). Further, when our replication results were adjusted for hypertension status, the identified variants remained significantly associated with atrial fibrillation (Supplementary Table 3). Ultimately, the development of a comprehensive risk score incorporating clinical, biochemical and genetic marker data will be necessary to clarify the incremental benefit of our findings in clinical care. Our eQTL analyses were limited to data available within the Genotype-Tissue Expression eQTL browser; future eQTL analyses in cardiac tissue may be helpful in identifying a relationship between the SNPs associated with atrial fibrillation risk and gene expression. Finally, we acknowledge that the identified variants may not be causal but may represent causal elements in the same or different molecular pathways; future statistical, bioinformatic and biological analyses investigating potential genetic interactions are warranted. Fine mapping and deep resequencing will be necessary to uncover the genetic architecture accompanying the identified common atrial fibrillation susceptibility signals.

In summary, our GWAS meta-analysis for atrial fibrillation has identified six new susceptibility loci in or near plausible candidate genes involved in pacemaking activity, signal transduction and cardiopulmonary development. Our results show that atrial fibrillation has multiple genetic associations and identifies new targets for biological investigation.

URLs

BIMBAM, http://quartus.uchicago.edu/~yguan/bimbam/index.html; Genotype-Tissue Expression eQTL browser, http://www.ncbi.nlm.nih.gov/gtex/GTEX2/gtex.cgi; iControlDB database, http://www.illumina.com/science/icontroldb.ilmn; IMPUTE, https://mathgen.stats.ox.ac.uk/impute/impute.html; MACH,

http://www.sph.umich.edu/csg/abecasis/MACH/; METAL,
http://www.sph.umich.edu/csg/abecasis/metal/index.html; PolyPhen-2,
http://genetics.bwh.harvard.edu/pph2/; SIFT, http://sift.jcvi.org/; SNAP,
http://www.broadinstitute.org/mpg/snap/ldsearch.php.

METHODS

Methods and any associated references are available in the online version of the paper.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Acknowledgments are contained in the Supplementary Note.

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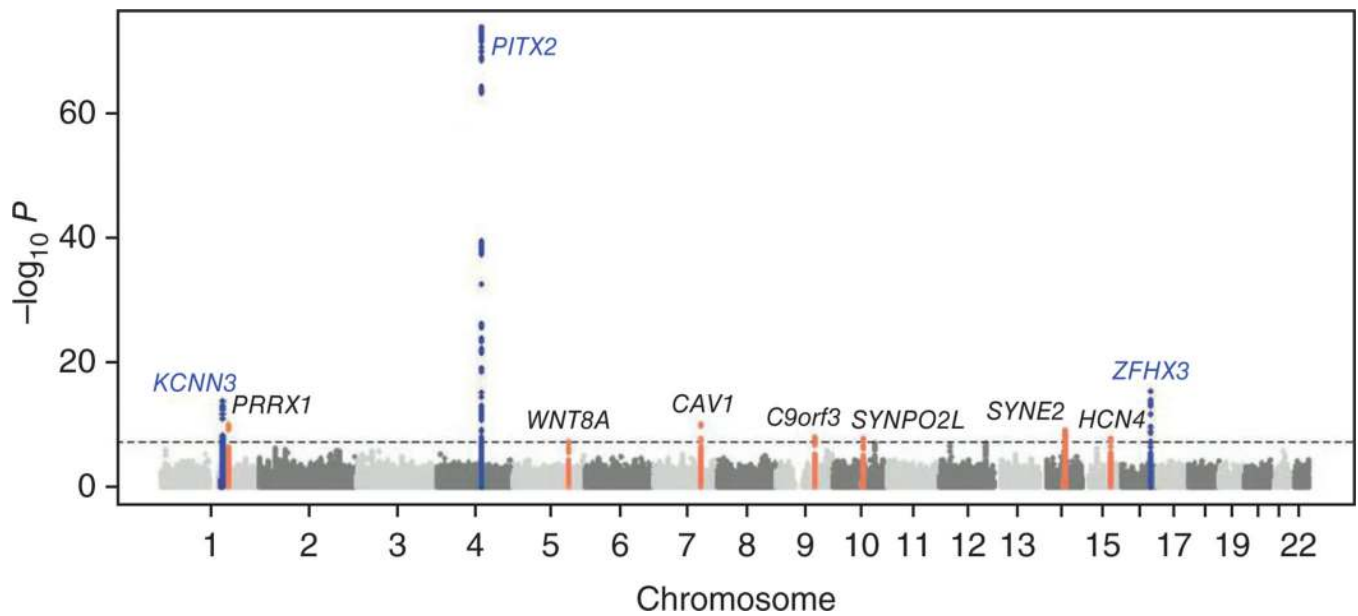
Figure 1.
Manhattan plot of meta-analysis results for genome-wide association with atrial fibrillation. The $-\log_{10}$ (P value) is plotted against the physical position of each SNP on each chromosome. The threshold for genome-wide significance, $P < 5 \times 10^{-8}$, is indicated by the dashed line. The three previously reported loci for atrial fibrillation are indicated in blue, and the seven new loci that exceeded the genome-wide significance threshold are indicated in orange.
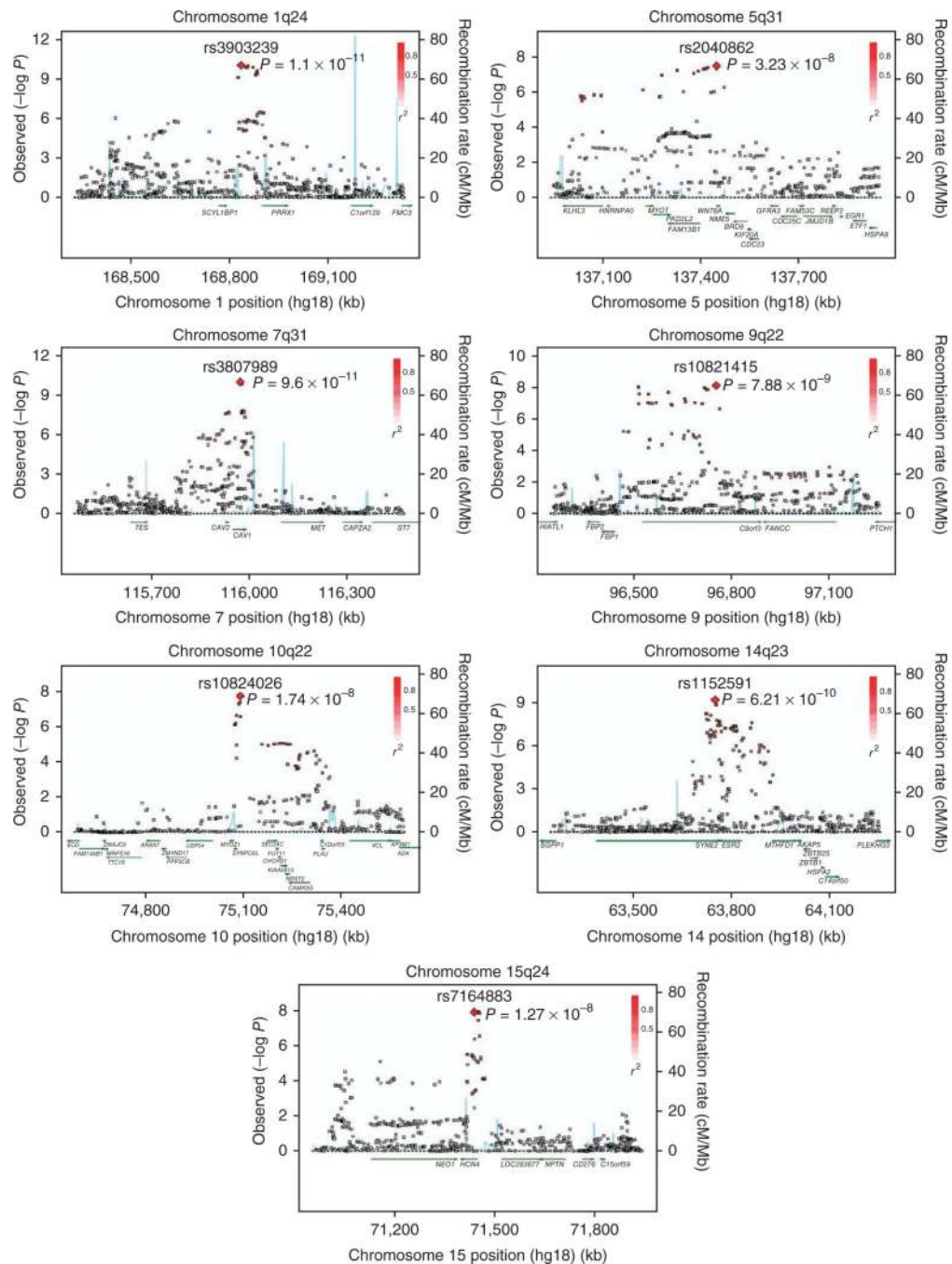
Figure 2.
Regional plots for seven new atrial fibrillation loci in the discovery sample with $P < 1 \times 10^{-8}$. SNPs are plotted by meta-analysis P value and genomic position (NCBI Build 36). The SNP of interest is labeled. The strength of LD is indicated by red coloring. Estimated recombination rates are shown by the blue peaks, and gene annotations are indicated by dark green arrows. LD and recombination rates are based on the Utah residents of Northern and Western European ancestry (CEU) HapMap cohort (release 22). Plots were prepared using SNAP[27].

Table 1

Subject characteristics

Cohort	Cohort type	Participants (n)	AF (n)	Males (%)	Age at DNA collection (mean ± s.d.)	Age at DNA collection (range)	Age of AF onset (mean ± s.d.)	Hypertension n (%)	Body mass index, in kg/m² (mean ± s.d.)	Diabetes n (%)	Myocardial infarction n (%)	Heart failure n (%)
Incident AF	Overall N											
ARIC	Cohort	8,890	802	4,181 (47.0)	54.3 ± 5.7	44–66	67.8 ± 6.8	2,376 (26.7)	27.0 ± 4.8	763 (8.6)	354 (4.0)	325 (3.7)
AGES	Cohort	2,959	158	1,154 (39.0)	76.5 ± 5.5	66–95	75.0 ± 8.8	2,595 (87.7)	27.1 ± 4.4	319 (10.8)	189 (6.39)	55 (1.9)
CHS	Cohort	3,204	764	1,242 (38.8)	72.2 ± 5.3	65–98	81.2 ± 6.0	1,678 (52.4)	26.3 ± 4.4	377 (11.8)	0	0
FHS	Cohort	4,062	310	1,771 (43.6)	64.7 ± 12.6	31–101	77.8 ± 10.6	2,001 (49.3)	27.7 ± 5.2	328 (8.07)	231 (5.7)	55 (1.4)
RS-I	Cohort	5,665	542	2,282 (40.3)	69.1 ± 9.0	55–99	77.6 ± 7.7	1,866 (32.9)	26.3 ± 3.7	567 (10.0)	632 (11.2)	156 (2.8)
WGHS	Cohort	20,836	648	0	54.1 ± 7.0	43–89	68.0 ± 8.2	5,022 (24.1)	25.9 ± 4.9	503 (2.4)	0	17 (0.1)
Prevalent AF	N per group											
AFNET	Cases	468	–	236 (50.4)	51.8 ± 7.2	29–74	51.3 ± 7.6	252 (53.8)	28.0 ± 4.9	36 (7.7)	6 (1.8)	14 (4.8)
KORA	Referents	438	–	219 (50.0)	56.2 ± 7.1	45–69	–	185 (42.2)	27.7 ± 4.5	37 (8.4)	6 (1.3)	13 (2.9)
AGES[a]	Cases	241	–	88 (55.7)	78.5 ± 5.9	67–95	80.9 ± 6.2	143 (90.5)	27.7 ± 4.4	20 (12.7)	8 (5.1)	5 (3.2)
	Referents	2,718	–	70 (36.1)	76.1 ± 5.4	66–94	80.4 ± 5.4	2,002 (78.2)	27.0 ± 4.5	269 (10.5)	122 (4.8)	27 (1.1)
CC	Cases	496	–	375 (75.6)	58.8 ± 10.7	20–84	51.7 ± 12.0	269 (54.2)	30.2 ± 6.2	28 (5.6)	0 (0)	0 (0)
	Referents	2,971	–	1,124 (37.8)	28.5 ± 22.2	0–87	–	–	–	–	–	–
HVH	Cases	95	–	28 (29.5)	59.5 ± 6.5	40–68	57.4 ± 6.4	50 (52.6)	34.1 ± 9.9	14 (14.7)	0	0
	Referents	193	–	106 (54.9)	59.5 ± 6.0	40–69	–	153 (79.3)	31.4 ± 7.2	31 (16.1)	9 (4.7)	7 (3.6)
CHS[a]	Cases	67	–	38 (56.7)	76.3 ± 5.8	66–90	–	35 (52.2)	26.6 ± 4.3	14 (20.9)	0	0
	Referents	3,204	–	1,242 (38.8)	72.2 ± 5.3	65–98	–	1,678 (52.4)	26.3 ± 4.4	377 (11.8)	0	0
FHS[a]	Cases	253	–	151 (59.7)	76.9 ± 9.9	45–97	70.9 ± 10.8	180 (71.1)	27.4 ± 4.8	41 (16.21)	60 (23.7)	57 (0.23)
	Referents	4,151	–	1,807 (43.5)	64.7 ± 12.6	31–101	–	2,036 (49.1)	27.7 ± 5.2	329 (7.9)	235 (5.7)	55 (1.32)
MGH	Cases	366	–	295 (80.6)	53.4 ± 10.5	21–77	46.1 ± 11.7	85.8 (22.7)	27.8 ± 5.0	12 (3.2)	4 (1.1)	10 (2.8)
MIGEN	Referents	911	–	485 (53.2)	47.9 ± 8.8	18–83	–	–	–	–	–	–
RS-I[a]	Cases	309	–	145 (46.9)	76.2 ± 8.7	56–98	–	131 (42.4)	25.9 ± 3.6	64 (20.7)	69 (22.3)	54 (17.5)
	Referents	5,665	–	2,282 (40.3)	69.1 ± 9.0	55–99	–	1,866 (32.9)	26.3 ± 3.7	567 (10.0)	632 (11.2)	156 (2.8)
SHIP	Cases	107	–	69 (64.5)	65.1 ± 11.5	21–81	–	59 (55.1)	29.6 ± 5.1	23 (21.5)	14 (13.1)	44 (41.1)
	Referents	1,816	–	906 (49.9)	50.7 ± 14.9	21–81	–	437 (24.1)	27.2 ± 4.5	131 (7.2)	54 (3.0)	157 (8.6)
Vanderbilt	Cases	1,081	–	738 (68.3)	59.5 ± 12.6	16–87	51.5 ± 14.7	625 (57.8)	30.7 ± 6.9	197 (18.2)	95 (8.8)	191 (17.6)

Cohort	Cohort type	Participants (n)	AF (n)	Males (%)	Age at DNA collection (mean ± s.d.)	Age at DNA collection (range)	Age of AF onset (mean ± s.d.)	Hypertension n (%)	Body mass index, in kg/m^2 (mean ± s.d.)	Diabetes n (%)	Myocardial infarction n (%)	Heart failure n (%)
	Referents	880	–	551 (62.6)	50.0 ± 17.4	18–91	–	463 (52.6)	28.0 ± 5.8	180 (20.5)	299 (34.0)	120 (13.6)

AF, atrial fibrillation.

[a] Studies included community-based prospectively ascertained prevalent atrial fibrillation cases. The other prevalent atrial fibrillation studies were of a case-control design.

Table 2

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

SNP	Locus	Closest gene	SNP location relative to closest gene	Minor/major allele	MAF (%)	Discovery			Replication		Overall	
						RR (95% CI)	Meta P value	I^2 (%), P value	RR (95% CI)	Meta P value	RR (95% CI)	Meta P value
rs6666258	1q21	KCNN3-PMVK	Intronic	C/G	29.9	1.18 (1.13–1.23)	2.0×10^{-14}	42.3, 0.04	–	–	–	–
rs3903239	1q24	PRRX1	46 kb upstream	G/A	44.7	1.14 (1.10–1.18)	9.1×10^{-11}	53.2, 6.3×10^{-3}	1.13 (1.06–1.20)	2.0×10^{-4}	**1.14 (1.10–1.17)**	8.4×10^{-14}
rs6817105	4q25	PITX2	150 kb upstream	C/T	13.1	1.64 (1.55–1.73)	1.8×10^{-74}	80.8, 1.4×10^{-10}	–	–	–	–
rs2040862	5q31	WNT8A	Intronic	T/C	17.8	1.15 (1.09–1.21)	3.2×10^{-8}	10, 0.34	1.04 (0.96–1.12)	3.6×10^{-1}	1.12 (1.07–1.17)	2.5×10^{-7}
rs3807989	7q31	CAV1	Intronic	A/G	40.4	0.88 (0.84–0.91)	9.6×10^{-11}	10, 0.34	0.93 (0.88–0.97)	2.7×10^{-3}	**0.90 (0.87–0.92)**	3.6×10^{-12}
rs10821415	9q22	C9orf3	Intronic	A/C	42.4	1.13 (1.08–1.18)	7.9×10^{-9}	49.5, 0.015	1.09 (1.04–1.15)	7.2×10^{-4}	**1.11 (1.08–1.15)**	4.2×10^{-11}
rs10824026	10q22	SYNPO2L	5 kb upstream	G/A	15.8	0.85 (0.81–0.90)	1.7×10^{-8}	37.9, 0.06	0.91 (0.83–0.99)	3.5×10^{-2}	**0.87 (0.83–0.91)**	4.0×10^{-9}
rs1152591	14q23	SYNE2	Intronic	A/G	47.6	1.13 (1.09–1.18)	6.2×10^{-10}	25.7, 0.16	1.12 (1.06–1.19)	1.9×10^{-4}	**1.13 (1.09–1.17)**	5.8×10^{-13}
rs7164883	15q24	HCN4	Intronic	G/A	16.0	1.16 (1.10–1.22)	1.3×10^{-8}	0, 0.85	1.24 (1.16–1.32)	1.3×10^{-10}	**1.19 (1.14–1.24)**	2.8×10^{-17}
rs2106261	16q22	ZFHX3	Intronic	T/C	17.6	1.24 (1.17–1.30)	3.2×10^{-16}	58.8, 1.6×10^{-3}	–	–	–	–

MAF, minor allele frequency; RR, relative risk. I^2 represents the proportion of variability in the effect size due to between-study variability. We did not attempt replication of the previously published genetic loci associated with atrial fibrillation on chromosomes 1q21 (KCNN3)[2], 4q25 (PITX2)[4] and 16q22 (ZFHX3)[3,5].