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REVIEW

Atrial fibrillation: the role of common and rare genetic variants

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Atrial fibrillation (AF) is the most common cardiac arrhythmia affecting 1–2% of the general population. A number of studies have demonstrated that AF, and in particular lone AF, has a substantial genetic component. Monogenic mutations in lone and familial AF, although rare, have been recognized for many years. Presently, mutations in 25 genes have been associated with AF. However, the complexity of monogenic AF is illustrated by the recent finding that both gain- and loss-of-function mutations in the same gene can cause AF. Genome-wide association studies (GWAS) have indicated that common single-nucleotide polymorphisms (SNPs) have a role in the development of AF. Following the first GWAS discovering the association between *PITX2* and AF, several new GWAS reports have identified SNPs associated with susceptibility of AF. To date, nine SNPs have been associated with AF. The exact biological pathways involving these SNPs and the development of AF are now starting to be elucidated. Since the first GWAS, the number of papers concerning the genetic basis of AF has increased drastically and the majority of these papers are for the first time included in a review. In this review, we discuss the genetic basis of AF and the role of both common and rare genetic variants in the susceptibility of developing AF. Furthermore, all rare variants reported to be associated with AF were systematically searched for in the Exome Sequencing Project Exome Variant Server.
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INTRODUCTION

Atrial fibrillation (AF) is a common supraventricular arrhythmia affecting 1–2% of the general population. The prevalence is increasing and is estimated to be doubled by 2040.[1,2] In most cases, AF is associated with cardiac risk factors such as hypertensive, ischemic and/or structural heart disease.[3,4] However, a subgroup of patients presents with AF in the absence of predisposing factors, a condition called 'lone AF', accounting for 10–20% of the total number of patients with AF.[5]

The mechanisms underlying AF are not fully understood but a heterogeneous model, based on the interaction of multiple substrates and triggers, is thought to underlie the pathophysiology of the disease. Lone AF has been suggested to be a primary electrical disease caused by disturbances in ionic currents and a genetic cause of these types of electrical disturbances is becoming increasingly recognized.[6]

A number of studies have demonstrated that AF and in particular lone AF have a substantial genetic component.[7–13] Oyen *et al*[14] have recently shown that an individual's risk of developing lone AF at a young age, increases drastically with both increasing number of relatives with lone AF and decreasing age at onset of the disease in these relatives, indicating an underlying genetic component in early onset lone AF.

Evidence for a heritable component of more common forms of AF has only recently been recognized. Fox *et al*[11] studied the inherited predisposition for AF in >5000 individuals and showed that, the development of AF in the offspring was independently associated with parental AF. Offspring of parents with AF had approximately a doubled 4-year risk of developing AF, even after adjusting for known risk factors such as hypertension, diabetes and myocardial infarction.

In this review, we discuss the genetic basis of AF and the role of both common and rare genetic variants in the susceptibility of developing AF.

MATERIALS AND METHODS

The review is based on an information retrieval carried out in Pubmed using the MeSH database and Google Scholar. A systematic literature search was performed to identify all studies published before April 2013, which investigated the genetic basis of AF. Searching with the query ((('Atrial fibrillation' (MeSH)) OR (atrial fibrillation)) AND ((('Genetics' (MeSH)) OR (genetic*)) AND ((('Mutation' (MeSH)) OR (mutation*) OR ('Polymorphism, single nucleotide' (MeSH)) OR (polymorphism, single nucleotide) OR (monogenic*) OR (GWAS)) yielded 527 articles. Only articles concerning the genetic basis of AF were included in this review. Small studies concerning common variants in genes not associated with AF were excluded, unless these studies have been replicated in other independent populations or convincing electrophysiology was presented, because of a high risk of false-positive associations. The reference list and related articles of each relevant publication were also examined to identify additional studies appropriate for inclusion in this literature study. Furthermore, rare variants associated with AF were systematically search for existence in NHLBI GO Exome Sequencing Project (ESP). The reference sequences used are: NM_000218 (*KCNQ1*), NM_000219 (*KCNE1*), NM_172201 (*KCNE2*), NM_005472 (*KCNE3*), NM_080671 (*KCNE4*), NM_012282 (*KCNE5*), NM_004980 (*KCND3*), NM_000238

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(*KCNH2*), NM_000891 (*KCNJ2*), NM_004982 (*KCNJ8*), NM_002234 (*KCNA5*), NM_005691 (*ABCC9*), NM_198056 (*SCN5A*), NM_001037 (*SCN1B*), NM_199037 (*SCN1Bb*), NM_004588 (*SCN2B*), NM_018400 (*SCN3B*), NM_153485 (*NUP155*), NM_000165 (*GJA1*), NM_005266 (*GJA5*), NM_006172 (*NPPA*), NM_002052 (*GATA4*), NM_005257 (*GATA6*), NM_005572 (*LMNA*), NM_004387 (*NKX2-5*) and NM_022469 (*GREM2*).

RESULTS

The role of common genetic variants

In recent years, genome-wide association studies (GWAS) have indicated that common single-nucleotide polymorphisms (SNPs) have a role in the development of AF. The first GWAS on AF showed that a SNP (rs2200733) located in proximity of the gene *PITX2* on chromosome 4q25 was highly associated with AF.[15] Since then, a number of GWAS have identified new SNPs associated with AF.[16–18] The GWAS linking these SNPs to AF were all performed in general AF populations and the biological pathway between the SNPs and the emergence of AF still remains to be solved.

In the following, current knowledge about the individual SNPs and their possible involvement in the pathogenesis of AF will shortly be presented (see Table 1).

The 4q25 locus

The SNP rs2200733 resides closest to the gene *PITX2* encoding the paired-like transcription factor PITX2. It was the first SNP to be identified and has been the top hit in all GWAS. Substantial research has therefore been focusing on this gene. In the human heart, *PITX2c* is the major isoform expressed[19] and is involved in the control of asymmetric cardiac morphogenesis.[15,20] Chung *et al*[21] have shown the association of a genetic variant on chromosome 4q25 with levels of *PITX2c* transcripts in left atrial tissue samples and knockout mouse models have indicated a critical role of the *PITX2*c in the development of the left atrium.[22] Of note, *PITX2c* was in a recent study demonstrated to be a requisite for the development of a sleeve of cardiomyocytes extending from the left atrium to the initial portion of the pulmonary veins, which is believed to be the anatomical substrate for AF and the fundament for pulmonary vein isolation when an ablation strategy is chosen in an AF patient.[23] In line with this, clinical and animal studies have demonstrated that ectopic foci of electrical activity arising from within the pulmonary veins and posterior left atrium have a substantial role in initiating and maintaining fibrillatory activity.[24,25] In contrast to studies indicating a structural role of *PITX2c*, a recently published study of heterozygous knockout (*PITX2c*$^{+/-}$) mice showed that these mice have a normal cardiac morphology and function, whereas expression of calcium ion binding proteins, gap- and tight junctions and ion channels was changed. They furthermore showed that isolated *PITX2c*$^{+/-}$ mice hearts were susceptible to AF during programmed pacing, because of shortening of atrial action potential durations (APDs) and the effective refractory period (ERP).[26] In line with these results, human studies have recently revealed that *PITX2c* expression is significantly decreased in patients with sustained AF, thus providing a molecular link between *PITX2* loss-of-function and AF.[27] Although a lot of studies indicate *PITX2* as having a role in AF, evidence regarding the expression of *PITX2* and its target genes in patients with AF is still missing. For instance, no studies regarding mRNA levels in atrial tissue of *PITX2* and target proteins measured by qPCR are available. For further insight and overview figures for the role of *PITX2* in AF pathogenesis, please see Liu *et al*[28] and Xiao *et al*.[29]

Table 1 Summary of SNPs associated with AF

SNP	Chromosome locus	Hg location	Closest gene	Location of SNP	Reference
rs2200733	4q25	4:111 710 169	PITX2	150-kb upstream	15
rs2106261	16q22	16:73 051 620	ZFHX3	Intronic	16
rs13376333	1q21	1:154 814 353	KCNN3	Intronic	17
rs3807989	7q31	7:116 186 241	CAV1/ CAV2	Intronic	18
rs3903239	1q24	1:170 569 317	PRRX1	46-kb upstream	18
rs1152591	14q23	14:64 680 848	SYNE2	Intronic	18
rs10821415	9q22	9:97 713 459	C9orf3	Intronic	18
rs7164883	15q24	15:73 652 174	HCN4	Intronic	18
rs10824026	10q22	10:75 421 208	SYNPO2L	5-kb upstream	18

All from GWAS.

Other GWAS loci

The SNP rs2106261 is located on chromosome 16q22 in an intronic region of transcription factor *ZFHX3*. The function of *ZFHX3* in cardiac tissue is unknown but it is expressed in mouse hearts.[30] The SNP rs13376333 is located on chromosome 1q21 in *KCNN3*, which encodes a calcium-activated potassium channel involved in atrial repolarization. In a rabbit burst-pacing model mimicking ectopic pulmonary vein foci, the pulmonary vein and atrial APD was shortened. This APD shortening was inhibited by apamin, a blocker of the calcium-activated potassium channels.[31] This provides a possible basis of *KCNN3* having a role in AF. The SNP rs3807989 is located close to the gene *CAV1*-encoding caveolin. *CAV1* is expressed in atrial myocytes and is necessary for the development of caveolae involved in electric signal transduction.[32] *CAV1* knockout mice develop dilated cardiomyopathy and pulmonary hypertension.[33] The SNP rs3903239 is located on chromosome 1q24, 46-kb upstream from the closest gene *PRRX1* encoding a homeodomain transcription factor highly expressed in the developing heart.[18] Knock-out mouse models have revealed that *PRRX1* is necessary for the normal development of great vessels and lung vascularization.[34,35] The SNP rs1152591 is located on chromosome 14q23, in an intron of the gene *SYNE2* encoding nesprin 2, which is a linker of nucleoskeleton and cytoskeleton (LINC) protein involved in maintaining cellular architecture and nuclear integrity.[36] It is highly expressed in heart and skeletal muscle, and mutations in *SYNE2* have been identified in families with Emery–Dreifuss muscular dystrophy. A disease that also displays cardiac manifestations characterized by cardiomyopathy and cardiac conduction defects.[18,37] The SNP rs10821415 is located in an open reading frame on chromosome 9. Genes near this locus is *FBP1* and *FBP2*, which is involved in gluconeogenesis.[18] How this SNP could be involved in developing AF remains unknown. The SNP rs7164883 is located on chromosome 15q24 in an intron of the gene *HCN4*, which encodes the cardiac pacemaker channel responsible for the funny current (I_f). The HCN4 channel is expressed in most of the conduction system and is the predominant isoform of the primary pacemaker in mouse hearts.[38] Mutations in *HCN4* have been associated with sinus node dysfunction.[39,40] The SNP rs10824026 is located on chromosome 10q22, 5-kb upstream of *SYNPO2L*.[18] Beqqali *et al*[41] identified the gene as encoding a cytoskeletal protein, which is highly expressed in the Z-disc of cardiac and skeletal muscle. They renamed it *CHAP*, cytoskeletal heart-enriched actin-associated protein. *CHAP* was found to have an important role in skeletal and cardiac muscle development.

Of note, Brugada *et al*[42] identified the *SYNPO2L* locus as a susceptibility locus for AF in a family with autosomal dominant AF.

None of the GWAS hits are found in amino-acid-coding regions of the genes. One possible explanation for the association with AF is that these variants may function as regulators of an adjacent gene, perhaps by altering the function of a promoter or enhancer and thereby causing an up or downregulation of genes nearby. Intense research is ongoing in order to correlate the GWAS hits with mRNA expression of genes located in proximity of the regions where the SNPs resides. These research projects are complicated by the fact that the top hits from GWAS are not necessarily the disease causative variants and other variants in high linkage must also be taken into account. Furthermore, it is possible that a GWAS hit is in high linkage disequilibrium (LD) with a low frequent variant, as was shown to be the case for a variant associated with sick sinus syndrome.[43] Thus, so far the mechanisms behind the association of the GWAS hits and AF still remain unresolved.

THE ROLE OF RARE GENETIC VARIANTS

Several genetic reports have revealed rare variants associated with AF in genes encoding cardiac gap junctions, signaling molecules, ion channels and accessory subunits.[6] Most of these studies show either gain- or loss-of-function mutations in the genes encoding proteins contributing to cardiac depolarization or repolarization leading to increased susceptibility of AF.

These results support the two current conceptual models for AF. The first one being that cardiac action potential (cAP) shortening functions as a substrate for re-entry wavelets in the atria.[44,45] The second one being that a prolonged ERP enhances the propensity for early afterdepolarization (EAD) and thereby, increasing the susceptibility to AF.[46] For a general introduction to cAP and involved ion channels, please see Nattel[44] and/or Shiroshita-Takeshita *et al*.[47]

In the following, current knowledge about rare variants in the individual genes and their possible involvement in the pathogenesis of AF will be presented.

POTASSIUM CHANNEL MUTATIONS

Chen *et al*[48] revealed the first association between mutations in *KCNQ1* and familial AF (see Table 2). The *KCNQ1* gene encodes the pore-forming α-subunit of the cardiac potassium channel I_{Ks} involved in cardiac repolarization. They studied a four-generation Chinese family with AF and indentified a mutation in all of the affected family members. Functional studies showed an increase in current density and altered gating and kinetic properties. This suggests a gain-of-function effect resulting in shortening of APD and reduction of the ERP.[6] A number of gain-of-function mutations in *KCNQ1* have been identified since (see Table 2).[49–53] Just recently, Bartos *et al*[54] identified a gain-of-function mutation in *KCNQ1* with high penetrance in five different families with early-onset AF. In addition to AF, several of the family members had abnormal QTc intervals, syncope or experienced sudden cardiac arrest or death.

The regulatory β-subunits of the I_{Ks} channel are encoded by five genes, *KCNE1–5*. In a recent study by Olesen *et al*[55] two non-synonymous mutations in *KCNE1* was associated with AF. In heterologous expression systems, both mutations showed significant gain-of-function for I_{Ks}. Yang *et al*[56] evaluated 28 unrelated Chinese kindred's with AF and identified a mutation in two probands in the *KCNE2* gene. The mutation showed a gain-of-function effect on I_{Ks}. Lundby *et al*[12] identified a mutation in *KCNE3* in a patient with lone AF and electrophysiological recordings displayed a gain-of-function effect on I_{Ks}. Recently, a mutation in *KCNE4* was identified by Mann *et al*[57] in a patient with AF. The functional consequence of this mutation is uncertain but the authors describe a possible change in I_{Ks}, I_{Kr} and I_{to}. Finally, Ravn *et al*.[13] found a missense mutation in *KCNE5* in 1 of 158 AF patients resulting in a gain-of-function effect on I_{Ks}.

The *KCNH2* gene encodes the α-subunit of I_{Kr}. A mutation in this gene was identified by Hong *et al*[58] in a family with both short QT and AF, which suggest an overlap in phenotypes. Previously, the mutation has been shown to drastically increase I_{Kr} and thus displaying gain-of-function.[59] Mann *et al*[57] have revealed additional mutations in *KCNH2* in AF patients (see Table 2).

The *KCNJ2* gene encodes the inward rectifier channel Kir2.1 that mediates the current I_{K1} involved in both the late phase of repolarization (phase 3) and the phase of maintaining resting membrane potential (phase 4). Xia *et al*[60] identified a missense mutation in *KCNJ2* in a Chinese kindred. Functional analysis of the mutant demonstrated a gain-of-function consequence on the Kir2.1 current.

Recently, Delaney *et al*[61] found a missense mutation in *KCNJ8* in a cohort of lone AF patients. The *KCNJ8* gene encodes the cardiac K_{ATP} channel Kir6.1. The Kir6.1 channel facilitates a non-voltage-gated inwardly rectifying potassium current, leading to a shortening of the APD under conditions of metabolic stress.[61] In a previous study, the mutation was shown to display a gain-of-function effect.[62]

The *KCNA5* gene encodes the atria-specific $K_v1.5$ channel responsible for the ultra-rapid delayed rectifier potassium current, I_{Kur}, involved in cardiac repolarization. Olson *et al*[63] identified a nonsense mutation in a familial case of AF. The mutation displayed a loss-of-function effect resulting in action potential prolongation and EAD. A number of mutations have since then been identified (see Table 2).[46,64–66]

The *ABCC9* gene encodes the SUR2A K_{ATP} channel subunit involved in maintaining electrical stability under stress, including adrenergic challenge. Olson *et al*[67] identified a missense mutation in the *ABCC9* gene in a female case with early-onset AF originating from the vein of Marshall. The mutation displayed loss-of-function.

SODIUM CHANNEL MUTATIONS

The *SCNA5* gene encodes the α-subunit of the cardiac sodium channel responsible for the I_{Na} current involved in cardiac depolarization (see Table 3). Darbar *et al*[68] identified rare variants in *SCN5A* in familial form of AF. Interestingly, several of the variants caused overlapping phenotypes with cardiomyopathy. Recently, Olesen *et al*[69] identified eight mutations in *SCN5A* in a cohort of lone AF patients. Functional investigations of the mutations revealed both compromised transient peak current and increased sustained current. These results indicate that both gain- or loss-of-function alterations in cardiac sodium current are involved in early-onset AF.

SCN1B-4B encodes the modifying β-subunits (Navβ1–β4) of the cardiac sodium channel. Watanabe *et al*[70] found two non-synonymous loss-of-function mutations in *SCN1B* and two in *SCN2B* in a cohort of 480 AF patients. Wang *et al*[71] found a loss-of-function mutation in *SCN3B* in another lone AF cohort. Moreover, Olesen *et al*[72] identified three non-synonymous mutations in *SCN3B*, which displayed a loss-of-function effect in the sodium current. One of the variants p.(Leu10Pro) has also been identified in a patient with Brugada syndrome (BrS).[73]

SCN1Bb encodes a second β1 transcript, named Navβ1B. Olesen *et al*[74] identified a missense mutation in *SCN1Bb* in two patients with lone AF as well as one patient with BrS. The same mutation was

Table 2 Summary of potassium channel rare variants associated with AF

Gene	Gene product	Nucleotide substitution	Amino-acid substitution	Documented family co-segregation	Electrophysiological consequence	Reference	Found in ESP EA minor/minor/ minor/major	Found in ESP AA minor/minor/ minor/major	Found in ESP all minor/minor/ minor/major
KCNQ1	α-Subunit of I_{Ks}	c.40C>T	p.(Arg14Cys)	Yes	Gain-of-function	53	0/0	0/0	0/0
		c.160_168ins ATCGCGCCC	p.(54_56insIleAlaPro)	Yes	Gain-of-function	52	0/0	0/0	0/0
		c.418A>G	p.(Ser140 Gly)	Yes	Gain-of-function	48	0/0	0/0	0/0
		c.421G>A	p.(Val141Met)	No	Gain-of-function	49	0/0	0/0	0/0
		c.625C>T	p.(Ser209Pro)	Yes	Gain-of-function	50	0/0	0/0	0/0
		c.692G>A	p.(Arg231His)	Yes	Gain-of-function	54	0/0	0/0	0/0
		c.693C>T	p.(Arg231Cys)	Yes	Gain-of-function	51	0/0	0/0	0/0
KCNE1	β-Subunit of I_{Ks}	c.74C>T	p.(Gly25Val)	No	Gain-of-function	55	0/0	0/0	0/0
		c.179G>A	p.(Gly60Asp)	Yes	Gain-of-function	55	0/0	0/0	0/0
KCNE2	β-Subunit of I_{Ks}	c.79C>T	p.(Arg27Cys)	Yes	Gain-of-function	56	0/0	0/0	0/0
KCNE3	β-Subunit of I_{Ks}	c.49G>A	p.(Val17Met)	No	Gain-of-function	12	0/0	0/0	0/0
KCNE4	β-Subunit of I_{Ks}	c.422A>C	p.(Glu141Ala)	?	Possible change	57	0/5	0/0	0/5
KCNE5	β-Subunit of I_{Ks}	c.193C>T	p.(Leu65Phe)	No	Gain-of-function	13	0/0	0/0	0/0
KCND3	Kv4.3	c.5C>A	p.(Ala2Glu)	?	No change	57	0/0	0/0	0/0
		c.641A>G	p.(Lys214Arg)	?	No change	57	0/4	0/0	0/4
		c.1633G>C	p.(Ala545Pro)	?	Gain-of-function	118	0/0	0/0	0/0
KCNH2	α-Subunit of I_{Kr}	c.526C>T	p.(Arg176Trp)	No	Loss-of-function	57,121	0/0	0/0	0/0
		c.1330G>A	p.(Glu444Lys)	Yes	Loss-of-function	57	0/0	0/0	0/0
		c.1764C>G	p.(Asn588Lys)	Yes	Gain-of-function	58,59	0/0	0/0	0/0
KCNJ2	Kir2.1	c.277G>A	p.(Val93Ile)	Yes	Gain-of-function	60	0/2	0/1	0/3
KCNJ8	Kir6.1	c.1265C>T	p.(Ser422Leu)	?	Gain-of-function	61,62	0/19	0/1	0/20
KCNA5	Kv1.5	c.143A>G	p.(Glu48Gly)	No	Gain-of-function	66	0/0	0/0	0/0
		c.211_243	p.(71_81del)	Yes	Loss-of-function	65	NA	NA	NA
		c.464A>G	p.(Tyr155Cys)	No	Loss-of-function	66	0/0	0/0	0/0
		c.913G>A	p.(Ala305Thr)	Yes	Gain-of-function	66	0/0	0/0	0/0
		c.964G>C	p.(Asp322His)	No	Gain-of-function	66	0/0	0/0	0/0
		c.1123G>T	p.(Glu375Ter)	Yes	Loss-of-function	63	0/0	0/0	0/0
		c.1407C>A	p.(Asp469Glu)	No	Loss-of-function	66	0/0	0/0	0/0
		c.1462C>T	p.(Pro488Ser)	No	Loss-of-function	66	0/0	0/0	0/0
		c.1580C>T	p.(Thr527Met)	Yes	Loss-of-function	46,64	0/0	0/0	0/0
		c.1703G>T	p.(Gly568Val)	Yes	Gain-of-function	57	0/3	0/0	0/3
		c.1727C>T	p.(Ala576Val)	Yes	Loss-of-function	46	0/0	0/0	0/0
		c.1828G>A	p.(Glu610Lys)	Yes	Loss-of-function	46	0/0	0/0	0/0
ABCC9	K_{ATP} channel	c.4640C>T	p.(Thr1547Ile)	?	Loss-of-function	67	0/0	0/0	0/0

Abbreviations: AA, African American; EA, European American; ESP, NHLBI GO Exome Sequencing Project; NA, data regarding major InDel is not available in EVS.

identified by Hu *et al*[75] in another BrS patient and functional data revealed that the mutation resulted in a 57% decrease in the peak sodium current while the Kv4.3 current was increased by 71% suggesting a combined effect with loss-of-function of the sodium channel current and a gain-of-function of the transient outward potassium current. On this basis, mutations in *SCN1Bb* could be susceptible variants for both AF and/or BrS.

NON-ION CHANNEL MUTATIONS

The *NUP155* gene encodes a nucleoporin, an essential component of the nuclear pore complex, a complex involved in nucleo-cytoplasmic transport (see Table 4). The gene is located on chromosome 5q13.[76] Oberti *et al*[77] mapped an AF locus to chromosome 5q13 in a large AF family with an autosomal recessive inheritance pattern. Subsequently, Zhang *et al*[78] revealed that the above mentioned locus was *NUP155* and identified a homozygous mutation in *NUP155* in all the affected family members. Heterozygous (*NUP155*$^{+/-}$) knockout mice showed AF phenotype.

NPPA encodes atrial natriuretic peptide, a circulating hormone produced in cardiac atria involved in the regulation of blood pressure through natriuresis, diuresis and vasodilatation.[79] Hodgson-Zingman *et al*[80] described a family with AF and an autosomal dominant pattern of inheritance. In this family, a heterozygous frameshift mutation in *NPPA* was detected and the mutation co-segregated with AF. The mutant peptide was shown to shorten atrial APD and the ERP in a rat heart model. Recently, Abraham *et al*[52] found a novel missense mutation in *NPPA* that was shown to co-segregate with early-onset AF. In the same cohort, the investigators found a *KCNQ1* mutation and functional analysis of the two mutations yielded strikingly similar I_{Ks} gain-of-function effects.

The *GATA4* and *GATA6* genes encode cardiac transcription factors. They work synergistically with *NKX2-5* in regulation of target gene expression, especially those involved in cardiogenesis.[81] Posch *et al*[82] found a *GATA4* mutation in one patient with familial lone AF in a lone AF cohort. A second mutation was found in a patient with sporadic lone AF. Additional studies have identified mutations in *GATA4* in different families, which co-segregated with AF and displayed decreased transcriptional effect.[83–85] Yang *et al*[86] reported two heterozygous *GATA6* mutations in two of 110 probands with familial AF. Each mutation co-segregated with AF transmitted as an autosomal dominant trait. The mutation was also associated with congenital cardiac defects in three AF patients in the families of the two probands. Additional studies have identified novel mutations in *GATA6* that co-segregated with AF and resulted in decreased transcriptional activity.[87,88]

The *LMNA* gene encodes lamin A/C, an intermediate filament protein associated with the inner nuclear membrane. Mutations in this gene have been associated with many diseases such as dilated cardiomyopathy and muscular dystrophy.[89] Beckmann *et al*[90] identified a heterozygous missense mutation in *LMNA* in a family with AF as well as SVT, VF, muscle weakness and sudden cardiac death. Just recently, Saj *et al*[91] found two variants in two unrelated probands with AF, one of them with episodes of AV-block, the other with reduced left ventricular contractile function (ejection fraction of 30%), left bundle branch block and family history of heart disease.

The *GREM2* gene encodes the bone morphogenetic protein (BMP) antagonist gremlin-2. Just recently, Müller *et al*[92] identified a variant

Table 3 Summary of sodium channel rare variants associated with AF

Gene	*Gene product*	*Nucleotide substitution*	*Amino-acid substitution*	*Documented family co-segregation*	*Electrophysiological consequence*	*Reference*	*Found in ESP EA minor/minor/ minor/major*	*Found in ESP AA minor/minor/ minor/major*	*Found in ESP all minor/minor/ minor/major*
SCN5A	α-Subunit of I_{Na}	c.414G>A	p.(Met138Ile)	Yes	Not investigated	68	0/0	0/1	0/1
		c.647C>T	p.(Ser216Leu)	?	Gain-of-function	68,122	0/11	0/1	0/12
		c.659C>T	p.(Thr220Ile)	No	Loss-of-function	69,122	0/4	0/0	0/4
\		c.1018C>G	p.(Arg340Gln)	No	Loss-of-function	69	0/0	0/0	0/0
		c.1127G>A	p.(Arg376His)	?	Not investigated	68	0/1	0/0	0/1
		c.1282G>A	p.(Glu428Lys)	Yes	Not investigated	68	0/2	0/0	0/2
		c.1333C>G	p.(His445Asp)	Yes	Not investigated	68	0/0	0/0	0/0
		c.1381T>G	p.(Leu461Val)	Yes	Not investigated	68	0/2	0/34	0/36
		c.1410C>G	p.(Asn470Lys)	Yes	Not investigated	68	0/0	0/0	0/0
		c.1441C>T	p.(Arg481Trp)	?	Not investigated	68	0/0	0/39	0/39
		c.1571C>A	p.(Ser524Tyr)	?	Not investigated	68	0/7	5/125	5/132
		c.1715C>A	p.(Ala572Asp)	Yes	Not investigated	68	0/20	0/1	0/21
		c.1852C>T	p.(Leu618Phe)	?	Not investigated	68	0/0	0/27	0/27
		c.1963G>A	p.(Glu655Lys)	Yes	Not investigated	68	0/0	0/0	0/0
		c.2989G>T	p.(Ala997Ser)	?	Gain-of-function	68,123	0/0	0/0	0/0
		c.3157G>A	p.(Glu1053Lys)	?	Not investigated	68	0/0	0/0	0/0
		c.3392C>T	p.(Thr1131Ile)	No	Not investigated	68	0/0	0/1	0/1
		c.3578G>A	p.(Arg1193Gln)	?	Gain-of-function	68,122	0/11	0/0	0/11
		c.3823G>A	p.(Asp1275Asn)	Yes	Loss-of-function	124	0/0	0/0	0/0
		c.3911C>T	p.(Thr1304Met)	No	Gain-of-function	69	0/4	0/1	0/5
		c.4478A>G	p.(Lys1493Arg)	Yes	Gain-of-function	125	0/0	0/0	0/0
		c.4786T>A	p.(Phe1596Ile)	?	No change	69	0/0	0/0	0/0
		c.4877G>A	p.(Arg1626His)	No	Combined	69	0/0	0/0	0/0
		c.5455G>A	p.(Asp1819Asn)	No	Combined	69	0/0	0/0	0/0
		c.5476C>T	p.(Arg1826Cys)	No	Not investigated	68	0/1	0/0	0/0
		c.5624T>C	p.(Met1875Thr)	Yes	Gain-of-function	126	0/0	0/0	0/0
		c.5689C>T	p.(Arg1897Trp)	No	Loss-of-function	69	0/3	0/0	0/3
		c.5851G>A	p.(Val1951Met)	Yes	Gain-of-function	68,69	0/0	0/0	0/0
		c.5958C>A	p.(Asn1986Lys)	Yes	Loss-of-function	127	0/0	0/0	0/0
		c.6010T>C	p.(Phe2004Leu)	?	Gain-of-function	68,122	1/24	0/2	1/26
SCN1B	β-Subunit of I_{Na}	c.254G>A	p.(Arg85His)	No	Loss-of-function	70	0/0	0/0	0/0
		c.457G>A	p.(Asp153Asn)	No	Loss-of-function	70	0/0	0/0	0/0
SCN1Bb	β-Subunit of I_{Na}	c.641G>A	p.(Arg214Gln)	No	Loss-of-function	74,75	0/21	0/2	0/23
SCN2B	β-Subunit of I_{Na}	c.82C>T	p.(Arg28Trp)	No	Loss-of-function	70	0/1	0/0	0/1
		c.83G>A	p.(Arg28Gln)	Yes	Loss-of-function	70	0/0	0/0	0/0
SCN3B	β-Subunit of I_{Na}	c.17G>A	p.(Arg6Lys)	No	Loss-of-function	72	0/0	0/0	0/0
		c.29T>C	p.(Leu10Pro)	No	Loss-of-function	72	0/0	0/1	0/1
		c.389C>T	p.(Ala130Val)	?	Loss-of-function	71	0/0	0/0	0/0
		c.482T>C	p.(Met161Thr)	No	Loss-of-function	72	0/0	0/0	0/0

Abbreviations: AA, African American; EA, European American; ESP, NHLBI GO Exome Sequencing Project.

in *GREM2* in two probands of a lone AF cohort. The variant was twofold more potent in antagonizing BMP than wild type. In a zebra fish model *GREM2* was shown to be required for cardiac laterality and atrial differentiation. *GREM2* over activity resulted in slower cardiac contraction rates and slower contraction velocity. Interestingly, *PITX2* (the top hit in GWAS) is regulated by BMP, suggesting that *GREM2* is acting upstream *PITX2*.

SOMATIC MUTATIONS

The *GJA1* and *GJA5* genes encode connexin43 and connexin40, respectively, which are gap-junction proteins in the atrial myocardium responsible for cell-to-cell conduction of the action potential (see Table 4). Thibodeau *et al*[93] identified a frameshift mutation, caused by a single-nucleotide deletion in *GJA1*, in atrial tissue in 1 of 10 unrelated lone AF patients. The mutation was absent from lymphocyte DNA of the patient indicating genetic mosaicism. The mutant protein demonstrated a trafficking defect leading to an intracellular retention of the protein and a failure of electric coupling between cells. Gollob *et al*[94] identified four heterozygous missense mutations in GJA5 in 4 of 15 idiopathic AF patients. Of note, in three of the patients the mutations were found to be somatic, suggesting that such mutations also could be involved in AF susceptibility. The mutant proteins revealed impaired intracellular transport or reduced intercellular electrical coupling. This may lead to conduction heterogeneity and re-entrant circuits. Recently, Christophersen *et al*[95] replicated the germline mutation in *GJA5*

Table 4 Summary of non-ion channel rare variants associated with AF

Gene	*Gene product*	*Nucleotide substitution*	*Amino-acid substitution*	*Documented family co-segregation*	*Electrophysiological consequence*	*Reference*	*Found in ESP EA minor/minor/ minor/major*	*Found in ESP AA minor/minor/ minor/major*	*Found in ESP all minor/minor/ minor/major*
NUP155	Nucleoporin	c.1172G>A	p.(Arg391His)	Yes	Loss-of-function	77,78	0/0	0/0	0/0
GJA1	Connexin43	c.932delC	Frameshift[a]	No	Loss-of-function	93	0/0	0/0	0/0
GJA5	Connexin40	c.113G>A	p.(Gly38Asp)[a]	No	Loss-of-function	94	0/0	0/0	0/0
		c.145C>T	p.(Gln49Ter)	Yes	Loss-of-function	97	0/0	0/0	0/0
		c.223A>T	p.(Ile75Phe)	Yes	Loss-of-function	98	0/0	0/0	0/0
		c.253G>A	p.(Val85Ile)	Yes	Not investigated	96	0/0	0/0	0/0
		c.262C>T	p.(Pro88Ser)[a]	No	Loss-of-function	94	0/0	0/0	0/0
		c.286G>T	p.(Ala96Ser)	No	Loss-of-function	94	0/0	0/0	0/0
		c.487A>G	p.(Met163Val)[a]	No	Loss-of-function	94	0/0	0/0	0/0
		c.661C>A	p.(Leu221Ile)	Yes	Not investigated	96	0/0	0/0	0/0
		c.685C>A	p.(Leu229Met)	Yes	Not investigated	96	0/0	0/0	0/0
NPPA	ANP	c.190A>C	p.(Ser64Arg)	Yes	Gain-of-function	52	0/24	0/2	0/26
		c.350C>T	p.(Ala117Val)	Yes	Not investigated	117	0/0	0/0	0/0
		c.456_457delAA	Frameshift	Yes	Gain-of-function	80	0/0	0/0	0/0
GATA4	Transcription factor	c.46G>T	p.(Gly16Cys)	Yes	Loss-of-function	84	0/0	0/0	0/0
		c.82C>G	p.(His28Asp)	Yes	Loss-of-function	84	0/0	0/0	0/0
		c.112T>G	p.(Tyr38Asp)	Yes	Loss-of-function	85	0/0	0/0	0/0
		c.209G>C	p.(Ser70Thr)	Yes	Loss-of-function	83	0/0	0/0	0/0
		c.307C>G	p.(Pro103Ala)	Yes	Loss-of-function	85	0/0	0/0	0/0
		c.479G>C	p.(Ser160Thr)	Yes	Loss-of-function	83	0/0	0/0	0/0
		c.1294C>T	p.(Met247Thr)	Yes	Not investigated	82	0/0	0/0	0/0
		c.1786C>T	p.(Ala411Val)	No	Not investigated	82	0/12	0/10	0/22
GATA6	Transcription factor	c.617A>C	p.(Gln206Pro)	Yes	Not investigated	86	0/0	0/0	0/0
		c.704A>C	p.(Tyr235Ser)	Yes	Loss-of-function	87	0/0	0/0	0/0
		c.795C>G	p.(Tyr265Ter)	Yes	Not investigated	86	0/0	0/0	0/0
		c.1406G>T	p.(Gly469Val)	Yes	Loss-of-function	88	0/0	0/0	0/0
LMNA	Lamin A/C	c.78C>T	p.(Ile26Ile)	?	?	91	0/2	0/0	0/2
		c.832G>A	p.(Ala278Thr)	Yes	Not investigated	90	0/0	0/0	0/0
		c.1462A>C	p.(Thr488Pro)	No	Not investigated	128	0/0	0/0	0/0
		c.1583C>T	p.(Thr528Met)	Yes	Not investigated	91	0/0	0/0	0/0
NKX2-5	Transcription factor	c.434T>C	p.(Phe145Ser)	Yes	Not investigated	117	0/0	0/0	0/0
GREM2	BMP antagonist	c.226C>G	p.(Gln76Glu)	No	Increase in inhibitory effect	92	0/27	0/4	0/31

Abbreviations: AA, African American; EA, European American; ESP, NHLBI GO Exome Sequencing Project.
[a]Somatic mutations.

(p.(Ala96Ser)), first detected by Gollob *et al*[94]. Yang *et al*[96,97] and Sun *et al*[98] have identified additional germline mutations in *GJA5* (see Table 4).

BIOINFORMATICS

With the recently published exome data from the NHLBI GO ESP, knowledge regarding genetic variation in the general population has become available. ESP currently holds genetic information on approximately 6500 (2200 African Americans and 4300 European Americans) unrelated individuals obtained by next-generation sequencing (NGS) of DNA from individuals recruited from different population studies.[99] No clinical data except from ethnicity were available in the ESP population, nor on request. Refsgaard *et al*[100–103] have recently conducted a number of studies that indicate that the exome database is representative for genetic variation in healthy subjects. All rare variants so far associated with AF (see Tables 2–4) and hypothesized to be important susceptibility variants in AF were systematically searched for in the ESP population. As illustrated by the last three columns in Tables 2–4, the vast majority of rare variants associated with AF were not present in the ESP population supporting that these variants are not random findings in original studies but indeed disease-causing or susceptibility mutations. If we make a(n) (arbitrary) cutoff point regarding prevalence of the variants in ESP at 0.5‰ of the total alleles corresponding to 1‰ of individuals (wary set) only p.(Ser422Leu) (*KCNJ8*), p.(Ser216Leu), p.(Leu461Val), p.(Arg481Trp), p.(Ser524Tyr), p.(Ala572Val), p.(Leu618Phe), p.(Arg1193Gln) and p.(Phe2004Leu) (*SCN5A*), p.(Ser64Arg) (*NPPA*), p.(Ala411Val) (*GATA4*) and p.(Gln76Glu) (*GREM2*) must be regarded as rare genetic variants less likely to be the major/monogenic cause of the disease. This is in contrast to four recently published articles concerning the prevalence in ESP of previously described mutations in genes involved in pathogenesis

of long QT syndrome (LQTS), sudden infant death syndrome (SIDS), cardiomyopathy and BrS. Here the investigators found a very high prevalence of previously described mutations in the ESP population. These findings question the disease-causing role of some of these variants in LQTS, SIDS, cardiomyopathy and BrS.[100–103]

GENETIC OVERLAP WITH OTHER CARDIAC DISEASES

There is a large overlap between different genes involved in arrhythmic diseases such as LQTS, BrS, short QT syndrome (SQTS), SIDS, cardiomyopathy and AF. The majority of genes associated with AF have been associated with other arrhythmic diseases. LQTS has been associated with mutations in *KCNQ1*,[104] *KCNE1-3*,[104,105] *KCNH2*,[104] *KCNJ2*,[104] and *SCN5A*.[104] BrS has been associated with mutations in *KCNE3*,[106] *KCNE5*,[107] *KCND3*,[108] *KCNH2*,[109] *SCN5A*,[110] *SCN1Bb*[74] and *SCN3B*.[111] Mutations in *KCNQ1*, *KCNH2* and *KCNJ2* have been associated with SQTS.[104] SIDS has been associated with mutations in *KCNQ1*, *KCNE1-2*, *KCNH2*, *KCNJ8*, *SCN5A*, *SCN1B*, *SCN3B* and *GJA1*.[101] Mutations in *ABCC9*,[112] *SCN5A*,[68] *NPPA*[113] and *LMNA*[102] have been associated with cardiomyopathy. Accordingly, nine genes associated with AF have not been associated with other arrhythmic diseases (*KCNE4*, *KCNA5*, *SCN2B*, *NUP155*, *GJA5*, *GATA4*, *GATA6*, *NKX2-5* and GREM2). A conclusion could be that these genes are special for AF. Another explanation is simply that the genes have only been investigated in AF cohorts.

Patients with genetically proven SQTS or LQTS have a higher risk of early-onset AF than the general population.[114,115] In a study by Johnson *et al*,[114] early-onset AF was observed in almost 2% of patients with genetically proven LQTS compared with the background prevalence of 0.1%. Interestingly, we recently found that both shortened and prolonged QTc interval durations are risk factors for incident AF, and that the association was strongest with respect to lone AF. Thus, the QTc interval does not simply seem to be a marker of cardiac disease but instead seems to be an inherent characteristic of an individual's cardiac electrophysiology. Thereby, indicating a link between AF and patients with extremes of QTc interval.[116]

EVIDENCE FOR INTERACTION BETWEEN COMMON AND RARE GENETIC VARIANTS

Although traditional linkage analysis and candidate gene approaches have revealed numerous suspected disease-causing mutations in familial AF, penetrance in these families is highly variable. Moreover, the transmission mode of other forms of AF, although studies suggest a high degree of heritability, remains unclear. This indicates that AF inheritance is complex and in many cases non-Mendelian.

A recent study by Ritchie *et al*[117] provide strong evidence that common genetic variants act as modifiers of rare genetic variants associated with familial AF. They studied whether the two previously reported common polymorphism in the chromosome 4q25 region[15] contribute to the variable penetrance of familial AF. DNA sequence analysis was performed for KCNQ1, KCNA5, NKX2-5, SCN5A and NPPA. They identified 11 families in which AF was present in ≥ 2 members and who also shared a mutation in one of the mentioned genes. Six families were found with rare variants in SCN5A, one with rare variants in NKX2.5, two with rare variants in NPPA, one with rare variants in KCNQ1 and one family with a rare variant in KCNA5. The penetrance of AF in probands carrying the putative mutation was low. However, the investigators found a significant interaction between common and rare genetic variants and onset of AF suggesting that addition of 4q25 genotypes helped predict, which carriers of the rare variants developed AF.

Another recent study by Mann *et al*[57] showed epistatic effects of potassium channel variation on cardiac repolarization and AF risk. The major cardiac K^+ channels were sequenced and 19 non-synonymous variants in 9 genes were found, 11 of them rare (6 of them novel). In all, 60 of the 80 AF probands had 2 or more variants. Individually, the variants had modest effect on potassium current but combinations of different variants showed both shortening end lengthening of APD suggesting a cumulative effect of ion channel variants both with regard to rare and common variants.

Olesen *et al*[118] just recently added further evidence for an interaction between common and rare genetic variants. In a lone AF patient, a mutation in *KCND3* was identified. Moreover, the patient was homozygous for the risk allele at both the *ZFHX3* (rs2106261) and *KCNN3* (rs13376333) loci, indicating that the patient was predisposed by common variants.

GENETIC TESTING IN AF

Ackerman *et al*[119] stated the recommendations for genetic testing in channelopathies and cardiomyopathies in a Heart Rhythm Society/European Heart Rhythm Society expert consensus document. Despite the high number of genes related to AF, the authors stated that genetic testing is not indicated for AF because none of the known disease-associated genes have been shown to account for $\geq 5\%$ of the disease. Moreover, although several SNPs have been associated with AF, little information links these specific genetic variants to distinct clinical outcomes for AF. However, a newly published article by Everett *et al*[120] added new knowledge regarding the link between SNPs and clinical outcome. The authors derived and validated a novel risk prediction model from 32 possible predictors in a cohort of 20 822 women without cardiovascular disease at baseline. A genetic risk score was created, which comprised the nine loci discussed in the section 'The role of common genetic variants'. The addition of genetic score to the AF risk algorithm model improved the c-index. This suggests that common variants in future could be used for risk stratification. Furthermore, genetic testing in AF could be an opportunity in near future with the implementation of NGS where whole genomes can be sequenced in few days.

Specific variants detected in patients, could potentially predict whether or not the patient will have a beneficial response to a specific drug. Thereby, genetic information may be used in personalized medicine in the future.

SUMMARY

In recent years, the evidence concerning the genetic basis of AF has been rapidly increasing. Many monogenic mutations or rare variants have been revealed by candidate gene approaches. Although useful in understanding the pathophysiology of AF, these mutations or rare variants are limited in explaining the heritability of AF because they only account for sporadic or familial cases of AF. GWAS are powerful in identifying new loci associated with an increased risk for development of AF. In the last 5 years, nine non-coding SNPs have been associated with increased risk of AF. These SNPs are believed to be signals for the causative genes. The genes in closest proximity to these SNPs have therefore been investigated and given new and valuable knowledge. However, the exact biological pathway between these non-coding SNPs and the emergence of AF still remains unsolved.

The new knowledge from the exome project and the implementation of NGS will hopefully make it possible to gain further insight to our understanding of AF.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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