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Genomics of Atrial Fibrillation

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Abstract Atrial fibrillation (AF) is a common clinical arrhythmia that appears to be highly heritable, despite representing a complex interplay of several disease processes that generally do not manifest until later in life. In this manuscript, we will review the genetic basis of this complex trait established through studies of familial AF, linkage and candidate gene studies of common AF, genome wide association studies (GWAS) of common AF, and transcriptomic studies of AF. Since AF is associated with a five-fold increase in the risk of stroke, we also review the intersection of common genetic factors associated with both of these conditions. Similarly, we highlight the intersection of common genetic markers associated with some risk factors for AF, such as hypertension and obesity, and AF. Lastly, we describe a paradigm where genetic factors predispose to the risk of AF, but which may require additional stress and trigger factors in older age to allow for the clinical manifestation of AF.

Keywords Atrial Fibrillation · Genomics · Stroke · Hypertension

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Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia seen in clinical practice. The etiology of AF is multifactorial, but includes a genetic predisposition to the arrhythmia. The road towards discovering the genetic basis of AF has progressed from identification of genes associated with familial AF to clinical observational studies demonstrating hereditability of common AF and to genome wide association studies (GWAS) that have identified to date 14 genetic loci associated with AF (Table 1) [1, 18, 30, 35, 43, 44, 46•, 47, 48•, 49–52, 53•, 54, 55]. However, identification of risk loci is only the start of a long process to discover the mechanisms by which these variants increase AF risk. Moreover, despite the identification of inherent genetic risk, we do not fully understand why AF typically does not manifest until later decades of life, nor why certain clinical factors, such as hypertension, obesity, male sex, or Caucasian ancestry, predispose to AF. Here, we will review the published evidence for the genetic basis of AF and discuss the results of recent genomic and transcriptomic analyses that may yield insights into some clinical observations of AF incidence.

Heritability of Atrial Fibrillation

The lifetime risk for developing AF is one in four for people older than 40 years [56]. Traditional risk factors for AF include older age, obesity, alcohol consumption, hypertension, left ventricular hypertrophy, heart failure, prolonged PR interval, and coronary artery disease [57]. The prevalence of AF has increased as a result of the aging and increased prevalence of obesity in the general population and in response to an increased awareness and diagnosis of the arrhythmia [57].

Table 1 Gene loci associated with atrial fibrillation

Gene	Function	Reference
Familial studies		
KCNQ1	Gain of function of potassium channel contributing to I_{ks}	[1–4]
KCNE2	Gain of function mutation of the potassium channel responsible for the I_{Ks} current	[5]
KCNH2	Encodes for the channel responsible for the rapidly depolarizing current I_{kr}	[6]
KCNJ2	Encodes for the inward rectifier potassium channel Kir 2.1	[7]
KCNA5	Modulation of ultrarapid depolarizing current I_{Kur}	[8]
SCN5A	Nav 1.5 responsible for upstroke of action potential	[9, 10]
SCN4B	β subunit of the voltage gated sodium channel	[11]
PRKAG2	ϒ2 subunit of AMP-activated protein kinase which regulates ATP generation and use.	[12]
NPPA	Frameshift mutation causes ANP to be resistant to breakdown increasing its half life	[13]
ABCC9	ATP binding cassette leads to loss of function of I_{KATP}	[14]
RYR	Alteration of ryanodine receptor leading to imbalance of calcium homeostasis	[15, 16]
NUP155	Nucleoporin 155, a component of the nucleopore formation reducing nuclear envelope permeability	[17]
LMNA	Laminin A/C in inner nuclear membrane	[18, 19]
GATA 4,5	Zinc finger transcription factor involved in cardiac development	[20–22]
Candidate gene studies		
KCNE 1,3,4 and *5*	Mutation of the β subunit of voltage gated potassium channel leading to altered function of I_{Ks}	[23–26]
KCNJ 5 and *8*	α subunits of inwardly rectifying potassium channels	[27, 28]
KCND3	$K_v4.3$ α subunit causing increase in the transient outward potassium current I_{to}	[29]
SCN1B,2B,3B	Mutation in the β subunits of the sodium channel leading to decreased sodium current	[30–32]
SCN10A	Nav 1.8 which participates in the late sodium current	[33, 34]
GJA5	Connexin 40 in gap junctions altering action potential propagation	[35–37]
NKX2.5	Homeobox transcription factor involved in cardiogenesis	[38, 39]
RAAS	Angiotensin conversion enzyme inhibitor, angiotensin gene promoter and angiotensinogen polymorphisms	[40–42]
Genome wide association studies		
PITX2	Right-left asymmetry, atrial cardiomyocyte and SA node development, intercalated disk	[43]
ZFHX3	Zinc finger homeobox 3; regulation of growth and differentiation of skeletal muscle and neuronal tissue.	[44, 45]
PRRX1	A homeodomain transcription factor expressed in connective tissue in the developing heart	[46•]
KCNN3	Intronic variant in the gene encoding for the calcium activated potassium channel SK3	[47]
HCN4	Hyperpolarization-activated cyclic nucleotide-gated 4 channel of the I_f	[46•]
CAV1	Caveolin-1, a structural component of caveolae	
SYNE2	Intronic SNP encoding for nespirin 2; anchors the nucleus to the cytoskeleton	[46•]
MYOZ1/SYNPO2L	Intergenic variant. *MYOZ1* encodes for myozenin 1 a protein involved in stabilizing the sarcomere	[46•]
C9orf3	Opening reading frame of chromosome 9, potential causal gene is still unclear	[46•]
GJA1	Connexin 43 in gap junctions altering action potential propagation	[48•]
NEURL	E3 ubiquitin ligase- interacts with *PITX2* and leads to increased AP duration	[48•]
CAND2	TBP- interacting protein involved in myogenesis	[48•]
TBX5	Transcription factor involved in the development of cardiac conduction system	[48•]
CUX2	Cut-like homebox 2 a transcription factor involved in neural development	[48•]

Despite the complex and aging-related clinical risk factors for the disease, AF is heritable. In the Framingham study, parental AF increased the risk of AF in offspring [58]. The odds ratio was 1.85 for AF in offspring who had one parent with AF and 3.23 when the sample was restricted to patients with parents who developed AF when they were <75 years old. A more recent study involving the Framingham cohort reported that new onset AF occurred more frequently in subjects with compared to without familial AF (adjusted hazard ratio 1.40; 95 % CI 1.13–1.74), independent of traditional AF risk factors [59]. In Iceland, Arnar et al. studied 5269 patients diagnosed with AF and age- and sex-matched controls [49]. The relative risk of AF in first-degree relative pairs was 1.77. When the analysis was restricted to first-degree relatives of patients with early onset AF (diagnosed <60 years of age), relative risk of AF was 4.67. Ellinor and colleagues

reported that lone AF (i.e., AF in the absence of structural heart disease) greatly increases the risk of AF in family members. A family history of AF was found in 38 % of patients with lone AF; the relative risk for sons was 8.1, daughters 9.5, brothers 70, and sisters 34 [60]. Lastly, a Danish twin study reported 22.0 % concordance rates for monozygotic pairs compared to 11.6 % for dizygotic pairs ($p < 0.0001$) with heritability of AF estimated to be 62 % [61]. Of note, such twin studies are of particular interest because they are less likely to lead to inflated estimates of heritability as a consequence of shared environmental risk factors.

Familial Monogenic AF

Families exhibiting a very high prevalence of AF have been reported as early as 1936 [62–64]. Traditional approaches to identifying genes responsible for AF in these presumably monogenic (or Mendelian) forms of AF include linkage studies and candidate gene searches. These studies have identified several ion channel gene mutations that were associated with familial AF, including potassium channel mutations (e.g., in *KCNQ1, KCNE2, KCNH2, KCNJ2, KCNA5*) for which gain-of-function mutations might be expected to promote AF by shortening of action potential duration and effective refractory periods, as well as in the sodium channel mutations (in *SCN5A* and *SCN4B*), which have also been associated with dilated cardiomyopathy [2–11].

Several non-ion channel genes have also been associated with familial monogenic AF, including *PRKAG2*, associated with familial Wolff-Parkinson-White syndrome and AF [12], and *NPPA*, which encodes for atrial natriuretic peptide [13]. Further, some developmental genes have been implicated, including genes encoding transcription factors involved in cardiogenesis from the *GATA* family [20–22] and *NKX2-5*, a homeobox-containing transcription factor involved in cardiac development and septation with mutations associated with atrial septal defect and AV conduction abnormalities [65]. Structural genes have also been associated with familial AF, including *GJA5*, which encodes for connexin 40 [66], a gap junction protein that is expressed in the atria and the conduction system and that is crucial for action potential propagation through myocytes, and *RYR2*, a gene which encodes the ryanodine receptor responsible for releasing intracellular stores of calcium [15, 16]. Finally, genes encoding nuclear membrane-associated proteins have been linked to familial AF. These include *LMNA*, which encodes for lamin expressed in the inner nuclear lamina. Mutations in *LMNA* have also been associated with muscular dystrophy, dilated cardiomyopathy, risk of sudden cardiac death [18, 19], and progeria syndrome. *NUP155* has also been associated with AF [17, 67]. This gene encodes for a nucleoporin that is involved in the assembly and functioning of the nuclear pore complex that regulates transport of macromolecules across the nuclear envelope between the cytoplasm and the nucleus [17, 67].

Polygenic Non-familial Common AF

Genetic Architecture

Although familial AF studies have contributed much to our understanding of the genes associated with monogenic forms of AF, they represent only a small fraction of the overall burden of disease. Overall, the genetics of common AF pathogenesis is complex, involving modest contributions to the risk of disease from genetic variations in many genes. The genetic architecture of a disease depends on the number of variants that influence a given pathology, the effect size of each variant on the phenotype, and the frequency of the variant. Complex diseases such as AF follow the "common disease common variant" hypothesis of genetic architecture where common variants with small effects appear to be responsible for a majority of the heritability of the trait [68].

Linkage and Candidate Gene Studies

Initial strategies to identify polymorphisms predisposing to common polygenic AF included linkage analyses and candidate gene-based association studies. Linkage analyses attempted to apply the same analytic techniques used to identify monogenic forms of disease to common polygenic forms of disease but proved to be inadequately powered to detect the modest effects of polymorphisms contributing to the risk of common AF [69].

Failed linkage studies were followed by candidate gene association studies, which relied on prior knowledge to generate hypotheses about possible polymorphisms that could have a causal relationship with a given disease and were limited by the potential bias of studying only what is already known [70]. Since these studies concentrated on only a small region of the genome, their pretest probability of identifying a real signal was low. The data published was further influenced by publication bias. A meta-analysis of 370 studies reporting on 36 different genetic associations illustrated this point well. The study found that, in 25 of 36 cases, the first study overestimated the replicated effect [69]. Further, 39 % of the genetic associations studied had significant heterogeneity between results [69]. Lack of replication for candidate gene association studies is common and can be a result of differences in study design, differences in studied populations with variable allele frequencies or relative risks of a certain variant, or due to real biological differences [70]. Furthermore, the polygenic nature of common and complex diseases involving many variants with small relative risks resulted in many

studies being substantially underpowered leading to a high rate of false-positive reports [69].

While as a whole candidate gene association studies have been disappointing, such studies nevertheless yielded some valuable insights into the potential mechanisms leading to AF. To begin, such studies implicated ion channel genes as contributants to risk of common AF, including those identified in familial studies. Potassium channel genes, including *KCNQ1, KCNH2, KCNJ5, KCNJ8, KCND3*, and *KCNE 1* through *5*, have been associated with AF risk [23–30]. The *KCNE* genes encode the beta subunits of voltage gated channels: *KCNE1* and *KCNQ1* contribute to the I_{Ks} current [24–26, 71]. Gain of function of I_{Ks} [24, 72] results in a faster repolarizing current and shorter effective refractory period, increasing the excitability of cells and susceptibility to arrhythmia. Loss of function prolongs the action potential and increases the likelihood of early after depolarizations [73]. *KCNA5* encodes for the α-subunit of the voltage sensor of a channel that generates the ultra-rapid potassium current, I_{Kur}, which is abundant in atria and detectable in failing ventricles. Hayashi et al. found a novel gain of function variant and a previously described *KCNA5* loss of function variant in a cohort of 72 patients with lone AF [30, 74].

Mutations in sodium channel subunit genes have also been associated with AF. The voltage-gated sodium channel consists of a pore-forming α subunit, encoded by the *SCNA* genes, and 4 different β subunits encoded by *SCN1B-4B*. This channel is responsible for the rapid influx of sodium ions that underlies the upstroke (phase 0) of the cardiac action potential. Several variants of *SCNA5*, which encodes the cardiac α-subunit of Nav1.5, have been reported in patients with common polygenic AF [75]. Gain-of-function mutations decrease the threshold voltage for the action potential and increase cellular excitability [76]. β-Subunits modulate the gating and voltage dependence of the channels, impact the trafficking of sodium channel to and from the cell membrane, and aid in cell adhesion. Mutations in the four β-subunits have been reported to attenuate sodium currents and shift the voltage dependence of sodium channel gating [31, 32]. A single nucleotide polymorphism (SNP) in *SCN10A*, which encodes Nav1.8, a voltage-gated sodium channel that participates in the late sodium current, was recently described to be associated with early onset AF [33].

Candidate gene analyses have also identified polymorphisms in non-ion channel genes with familial AF. Implicated genes include those encoding connexin 40, *GJA5* [35–37, 77], the transcription factor gene *NKX2.5* [38, 39], and the *LMNA* gene [78]. Genes involved in the renin-angiotensin-aldosterone pathway, including the angiotensin-converting enzyme, angiotensin gene promoter, and angiotensinogen [40–42, 79], have also been associated with AF.

Identification of AF-Associated Genetic Loci Through Genome Wide Association Studies

Genome wide association studies (GWAS) represent a relatively new but important study design to overcome the inherent bias of candidate gene analyses. By comparing the frequencies of common genetic variants among subjects with and without a disease or level of quantitative trait, GWAS has been able to identify hundreds of novel genetic associations over the last 10 years involving common single nucleotide polymorphisms (SNPs). GWAS analyze hundreds of thousands to millions of SNPs concurrently. To correct for multiple testing, thresholds for significance typically need to be adjusted to a p value of $<5X10^{-8}$, which effectively corrects for one million independent tests. Once a SNP or a set of SNPs in a region reaches genome wide significance, the next important step is to link the SNP association to a causal gene. Generally, this process begins with an assessment of the genes that are in proximity or within the susceptibility locus and should not be deemed certain until a functional mechanism has been fully worked out in detail through multiple additional experiments [80].

Developmental Genes

In 2007, the first GWAS for AF identified a susceptibility locus at chromosome 4q25 in a population of European descent and replicated this association in an Asian population [43]. This association has been further replicated in all AF GWAS, to date [81–83]. Further, this locus was associated with recurrence of AF after ablation and new onset AF in the postoperative period after coronary bypass surgery [50, 81].

Additional GWAS have identified at least 4 independent loci at chromosome 4q25 for AF [84]. As is often the case, the lead SNPs in this region are in non-coding regions. Nevertheless, the closest gene is the paired-like homeodomain 2 *PITX2* gene, located in a linkage disequilibrium (LD) block upstream to the susceptibility locus. *PITX2* is involved in embryogenesis and left-right differentiation of the heart. *PITX2* encodes 3 different isoforms of the protein by alternative splicing. Importantly, the Pitx2c isoform is mainly found in the left atrium and pulmonary veins [85, 86]. In Pitx2c conditional knockout mice, Pitx2c-deficient mice do not develop a pulmonary myocardial sleeve, and Pitx2 appears to suppress a left atrial sinus node program [87]. Compared to wild-type mice, Pitx2null+/− and Pitx2null−/− mice heterozygous and homozygous for a Pitx2-null allele that removes all Pitx2 isoform function showed step-wise increased expression of *HCN4*, which encodes a hyperpolarization-activated cyclic nucleotide-gated channel contributing to pacemaker currents, as well as *TBX*3 and *SHOX2* that are also required for sinoatrial node development [86]. Expression of *KCNQ1* was also

increased [87], which would result in a shorter effective refractory period, facilitating action potential triggering and conduction. Mouse *PITX2* conditional or heterozgygote mutation models have shown alterations in ion channel, calcium handling, gap and tight junction gene expression [88–91], *PITX2* regulation of miRNAs [90–92], and atrial arrhythmia inducibility [87, 88, 90]. Thus, *PITX2* is a biologically attractive candidate causative gene in the region, as it appears to be involved in the regulation of several ion channel genes that plausibly may predispose to atrial arrhythmia, it directs asymmetric morphogenesis of the heart, is involved in the formation of pulmonary veins, the putative site of AF initiation targeted by clinical AF ablation [93], and appears to repress a left atrial sinus node program, genetic variation of which might contribute to electrical triggers recorded within pulmonary vein ostia.

The expression of *PITX2* has been shown to markedly decrease postnatally. The mechanisms leading to postnatal inactivation of *PITX2* are actively being researched and include possible mediation by various risk factors, such as pressure and volume overload and oxidative stress that come with age. Tao et al. showed that postnatal inactivation of *PITX2* leads to abnormal R-R intervals and to differential expression of genes involved in cell junction organization, including the intercalated disk, ion channels, and caveolae [94].

GWAS of AF have also identified several other loci associated with transcription factors that appear to be important for cardiac development. One of these is located on chromosome 16q22 intronic to the zinc finger homeobox 3 gene (*ZFHX3*), known as AT motif-binding transcription factor 1(*ATBF1*) [44], which is involved in regulation of growth and differentiation of skeletal muscle and neuronal tissues [44]. *ZFHX3* is required for the transcriptional activation of the gene POU class I homebox 1 (*POU1F1*), which interacts with *PITX2*, enabling DNA binding and transcriptional activity. This might be a mechanism by which it predisposes to AF [83]. Later, another GWAS described *PRRX1* which encodes for a homeodomain transcription factor expressed in connective tissue in the developing heart [46•]. In animal models, knockout of *PRRX1* led to abnormalities in the pulmonary vasculature [95].

Ion Channels and Pores

Thus far, two loci in ion channel genes have been significantly associated with AF in GWAS studies. First, a locus was identified on 1q22 within an intron of the *KCNN3* gene, which encodes for the calcium-activated potassium channel SK3 [47]. SK3 contributes to the repolarization phase of the action potential [96]. Second, a locus on chromosome 15q24 within an intron of the hyperpolarization-activated cyclic nucleotide-gated 4 (*HCN4*) gene was described [46•]. *HCN4* is expressed in the sinoatrial (SA) node and underlies the I_f current normally responsible for the pacemaker current in nodal myocytes. Mutations of this channel may lead to diminished action potential frequency (heart rate slowing) and delayed after depolarizations that might trigger AF [97]. As discussed above, Pitx2 represses Hcn4 expression in the left atrium [86, 87].

Structural Genes

A meta-analysis of GWAS in 2010 identified several new AF loci, including three in/near genes involved in cellular structural components. Cell membranes contain invaginations, known as caveolae, that participate in cell transport. A locus on chromosome 7q31 within an intron of the gene *CAV1*, which encodes caveolin-1, was associated with AF [46•]. *CAV1* is co-expressed with a potassium channel gene (*KCNH2*), which is involved in the repolarization phase of the action potential. The risk allele and AF are associated with decreased expression of *CAV1* [98]. Further, caveolin-1 has been postulated as an anti-fibrotic element in atrial tissue since down-regulation of *Cav-1* by siRNA leads to increased TGF-β1-induced activation of the Smad signal pathway and collagen production [99].

An intronic SNP located on chromosome 14q23 in the gene *SYNE2*, which encodes for nesprin-2, has also been associated with AF. Nesprin-2 is located in the outer nuclear membrane and sarcomere and anchors nuclei to the cytoskeleton. A large meta-analysis of GWAS by Ellinor, et al. identified a susceptibility locus between two genes, *SYNPO2L* and *MYOZ1*, on chromosome 10 and another locus in the an open-reading frame on chromosome 9 (*C9orf3*) [46•]. Myozenin-1 is a protein involved in stabilizing the sarcomere, encoded by *MYOZ1*. *C9orf3* region is associated with three coding genes, including *FBP1* and *FBP2*, which are involved in gluconeogenesis [46•].

Gene mRNA Expression studies

Although GWAS studies identify regions in the genome that are associated with a phenotype, the lead SNP within a susceptibility locus is often not the causal variant. Furthermore, the lead SNP is often not within a coding region of a gene and usually near several genes, making it challenging to determine which gene in the region is the causal gene. One way to identify with more certainty the causal gene is to leverage gene expression studies to determine whether a lead SNP is preferentially associated with the level of mRNA of one or more genes nearby. SNPs that are associated with mRNA are often termed expression quantitative trait loci (eQTLs) [100]. As the control of gene expression is often tissue-specific, assessment of relationships between SNPs and mRNA expression among circulating cells in blood may be inadequate to assess the

effects of SNPs on the expression of genes predisposing to AF. Gene expression studies involving more relevant target tissues, such as the left atrium itself, may substantially increase the yield of identifying causal genes among susceptibility loci of AF.

Genetic variants can change the expression of mRNA and proteins by *cis* or *trans* mechanisms. *Cis*-acting variants affect expression of genes nearby on the same chromosome as the gene variants, while *trans*-acting variants can act on genes that are far from them. Such transcriptional studies can help guide the identification of the genes associated with genetic variants identified by GWAS.

eQTL analysis of the AF-associated locus in between *SYNPO2L* and *MYOZ1* has demonstrated an association between the high risk alleles in this region and decreased expression of *MYOZ1*, implicating *MYOZ1* as the most likely causal gene associated with this AF locus [98, 101]. In another eQTL study predominantly involving right atrial appendage tissues, AF risk alleles were associated with increased expression of *PITX2a* and decreased expression of *MYOZ1, CAV1, C9orf3*, and *FANCC* [98].

Despite the finding of cis-eQTLs for genes noted above, significant eQTLs for the top GWAS locus from AF on chromosome 4q25 were not found for *PITX2c* in human adult left atrial appendage tissues [102]. Recently, we performed RNA sequencing on human left/right atrial tissue pairs and reported the presence of an intergenic long non-coding RNA sequence adjacent to the *PITX2* gene (PANCR) differentially expressed in the left atria and eye, but no other heart chambers. Expression of PANCR RNA correlated with *PITX2c* mRNA expression. In human embryonic stem cells, both PANCR and *PITX2c* RNAs were coordinately expressed during early differentiation of the stem cells towards cardiomyocytes. siRNA-mediated PANCR knockdown decreased *PITX2c* expression and resulted in similar RNA expression profiles as *PITX2c* knockdown cells, whereas *PITX2c* knockdown did not affect PANCR expression, suggesting that PANCR is an important regulator of *PITX2c* expression [103]. However, similar to *PITX2c* in human adult left atrial appendage tissue, chromosome 4q25 risk SNPs for AF were not associated with PANCR or *PITX2c* expression [102, 103]. These findings may be a consequence of *PITX2* and PANCR being most active during embryologic development in the left atrium or in the tissue within the pulmonary vein–left atrial junction which may have some distinct differences with the tissue making up the adult left atrial appendage.

A combination of large-scale GWAS, cis-eQTL, and functional evaluation led to the identification of 5 novel loci associated with AF near the genes *NEURL, GJA1, TBX5, CAND2*, and *CUX2* [48•]. AF-associated cis-eQTLs were identified for *CAND2, GJA1*, and *TBX5. NEURL* encodes for an E3 ubiquitin ligase that was found to co-localize with *PITX2*. Another signal was found in a SNP intronic to *TBX5*, a transcription factor implicated in conduction abnormalities, and in *CAND2* [48•], which encodes for a TATA-binding protein transcription factor involved in myogenesis [104]. eQTL analysis of *CAND2* and *TBX5* showed that the risk alleles were associated with an increased expression of these genes. Additional functional studies demonstrated that *NEURL* and *CAND2* knockout zebrafish models had longer atrial action potential duration [48•]. Propagation of the action potential through myocytes requires a balanced expression of two gap junction proteins: connexin 40 and 43 [105]. Cis-eQTL associations showed the AF risk allele at the *GJA1* locus was associated with lower expression of *GJA1*, which encodes connexin 43 [48•]. Connexin abnormalities can lead to reduced conduction velocity and action potential propagation. In the same paper, a novel SNP located within an intron of the *CUX2* gene was found to be associated with AF only in a Japanese population [48•]. *CUX2* was recently associated with increased oxidative DNA damage in embryonic neurons [106]. However, a significant cis-eQTL was not identified for the risk allele.

Transcriptomic Analyses and the Age Paradox in AF

Despite the association of several developmental genes with AF risk, AF typically does not manifest until decades after birth. To gain insight on this paradox, we recently performed a gene expression study of left atrial appendage tissue from 207 adults with AF and 32 adults without AF to compare gene expression in 3 groups based on AF history and rhythm at the time of resection of the atrial appendage: (1) no AF history and in sinus rhythm (SR/SR), (2) AF history and in sinus rhythm (AF/SR), and (3) AF history and in AF rhythm (AF/AF) [53•]. We identified differentially coexpressed gene modules between the SR/SR and AF/SR groups ("AF susceptibility" comparison) and between the AF/SR and AF/AF groups ("AF activity or persistence" comparison). Gene set enrichment analyses showed that AF susceptibility was associated with decreased transcriptional responses to cellular stress, inflammation, oxidation, and unfolded proteins, while AF persistence was associated with changes in ion channel expression and decreased stress responses. These findings may help explain the AF age paradox, whereby AF does not manifest until there is impaired responsiveness to persistent cellular stress and aging, decades after the GWAS-identified AF loci exert their activity during cardiac and pulmonary vein development.

The reduced cellular stress response to protein unfolding is of particular interest, as this geneset includes targets of several transcription factors such as the CREB/ATF family, HSF1, ATF6, SRF, E2F1, and SP1 [53•]. Alterations in many of these transcription factors can lead to abnormal proteostasis and deposition of unfolded amyloid proteins. Deposition of isolated atrial amyloid is well described and associated with older

age and the presence of AF [107, 108]. Interestingly, atrial amyloid has also been reported to be more frequent in left than in right atrial appendages [108]. Isolated atrial amyloid protein has been shown to be due to aggregates of atrial natriuretic peptide (ANP) [107, 109], and expression levels of *NPPA*, which encodes for ANP, are high in adult human atrial appendage tissues. Expression of *NPPA* was up-regulated in Pitx2 heterozygote and null mice [86]. Further, a recent study in a Chinese population suggested that *TBX5* mutations increases the expression of ANP and connexin 40 [110]. Senile accumulation of the protein aggregates may contribute to the predisposition to AF with aging.

AF Genetics and Stroke

One of the most devastating complications of AF is stroke. Conventional risk factors account for only 60 % of the variation in the risk of stroke [111]. Similar to AF, stroke has a genetic predisposition. Further, risk factors differ depending on the etiology of the stroke, which includes hemorrhagic, ischemic, cardioembolic, large artery due to atherosclerosis, small vessel, and cryptogenic forms [112]. Genetic studies have attempted to elucidate new stroke prediction, prevention, and treatment approaches and have led to the discovery of common genetic variants associated with both AF and stroke (Table 2, Fig. 1).

Soon after publication of the first GWAS of AF implicating SNPs in the 4q25 region, the same SNPs were also identified as risk factors for ischemic stroke. Gretarsdottir et al. showed that rs2200733, one of the first 4q25 SNPs demonstrated to be strongly associated with AF risk, was associated with ischemic stroke, and even more strongly with cardioembolic stroke [115]. This association was further replicated in a targeted sequencing study of a Polish population [114]. Lastly, another study showed a different locus in the same region was associated only with cardioembolic stroke [113].

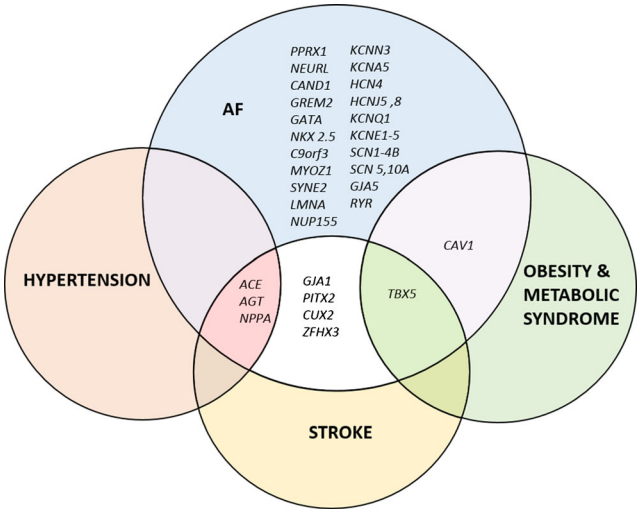

Fig. 1 Common implicated genes associated with AF, stroke, hypertension, and obesity/metabolic syndrome

Large-scale studies have found similar genetic associations between AF and stroke. Gudbjartsson et al. reported a case control genomic study, finding that the SNP rs7193343 at the *ZFHX3* locus was associated with ischemic and cardioembolic stroke [45]. Several GWAS studies have confirmed this association, including the METASTROKE study, which incorporated about 70,000 subjects. These studies confirmed that the *PITX2* and *ZFHX3* SNPs were associated with ischemic stroke and more specifically with cardioembolic stroke, even though only half of their population was sub classified by type of stroke [116]. A recent exome sequencing study

Table 2 Common variants between AF and stroke

Reference	Number of patients	Nearby gene-SNP variant	Association
Lemmens et al. [113]	4199 patients with IS	*PITX2*-rs1906591	AF and IS of CES etiology
Wnuk et al. [114]	729 Polish patients	*PITX2*-rs2200733	CES related to AF
Gretarsdottir et al. [115]	1661 Icelandic patients	*PITX2*-rs2200733	CES
Taylor et al. [116][a]	Meta-analysis including 74,393 patients	*PITX2*-rs6843082	CES
Gudbjartsson et al. [45][a]		*ZFHX3*-rs7193343	IS and CES
Sinner et al. [48•][a]	Meta-analysis and metastroke 74,393 subjects	*CUX2*-rs6490029	IS
		GJA1-rs13216675	CES
		TBX5-rs10507248	CES
Renin angiotensin aldosterone system			
Tsai et al. [117]	712 AF patients	rs5049/rs5051 (G217/G6) allele in AGT promoter gene	stroke
Stetskaia et al. [118]	638 patients	rs4762 (T174M)	Stroke in females

AF atrial fibrillation, *AGT* angiotensin, *CES* cardioembolic stroke, *LA* left atrium, *IS* ischemic stroke

[a] GWAS studies

replicated the *ZFHX3* locus association with ischemic stroke [119]. Using the METASTROKE collaboration data, an association between ischemic stroke with an AF-risk SNP intronic to *CUX2* was found, though the AF risk allele was paradoxically associated with a decreased the risk of stroke (OR 0.95; 95 % CI 0.91–0.98). Sub-analysis by type of stroke found an association of *GJA1* and *TBX5* AF-risk SNPs with cardioembolic stroke [48•].

The mentioned associations are likely due to an enrichment of AF cases among ischemic and cardioembolic stroke cases. They might be markers of AF as a predominant cause of embolic rather than non-embolic stroke. Studies have not consistently classified strokes, and the lack of differentiation by subtype of stroke reduces power for stroke subtype analyses. Without a large study of isolated cardioembolic stroke, signals attributed to ischemic stroke are an aggregate of multiple mechanisms leading to stroke. Nevertheless, these genetic variants might ultimately prove useful in differentiating the etiology of an ischemic stroke and in identifying patients with cryptogenic stroke who are most likely to have underlying undiagnosed AF. These patients may warrant more monitoring and potential anticoagulation for secondary prevention of stroke.

Current stroke prevention scores rely on clinical data to determine therapy, but adding genetic markers may improve the predictive value of these tools. Tada et al. constructed a 12 SNP genetic risk score (including SNPs near *PITX2, KCNN3, PRRX1, CAV1*, C9orf3, *SYNPO2L, SYNE2, HCN4*, *ZFHX3*) to identify people at higher risk for AF and found that the score in addition to the CHADS2 score led to better prediction of ischemic stroke in AF patients [120•]. The renin angiotensin aldosterone (RAAS) system plays an important role in inflammation, hypertension, tissue remodeling, and fibrosis. A preliminary association of the G6 polymorphism (a polymorphism located in the promoter region of the angiotensin gene leading to increased angiotensinogen production) has been found in stroke in two candidate gene studies [117, 121] and in one candidate gene study of AF patients [42]. Tsai et al. showed that the addition of the G6 polymorphism to the CHADS2 score improved the ability to predict stroke in an AF population [122]. Other elements of the RAAS system, such as the angiotensinogen gene and angiotensinogen-converting enzyme gene, have also been associated with AF and stroke in candidate gene analyses [40, 41, 118, 123].

Inflammation likely plays a role in both stroke and AF. Ischemic brain injury is characterized by inflammation. However, data regarding a polymorphism in the CRP gene, which has been reported in candidate gene analysis as an independent risk factor for stroke, have been conflicting [124, 125]. Other components of the inflammatory cascade studied include IL-1a, TNFα, IL-10, ICAM-1, though evidence has again been inconsistent [126–130] with studies limited by heterogeneity and small sample size.

Risk factors for AF: Hypertension, Obesity, and Common Genetic Variants

Hypertension is an important modifiable risk factor for AF [131]. While GWAS for hypertension have not identified common SNPs that have also been associated with AF, several genetic variants associated with hypertension may contribute to AF.

The genetic architecture of blood pressure appears to be extremely complex with ~100 independent variants accounting for only around 2 % of the variance in blood pressure [132]. These include loci near *NPPA* and *NPPB*, which encode precursors of the natriuretic peptides. Polymorphisms in these loci correlate with the level of circulating natriuretic peptide and blood pressure [132, 133]. AF has been associated with variants in the *NPPA* gene [134, 135], causing resistance to ANP breakdown and increasing its half-life [13]. Atrial amyloid is largely derived from ANP. Transcriptomic differential gene expression analysis (discussed above) showed that AF susceptibility is associated with decreased targets of cellular stress response to protein unfolding [53•], leading to abnormal proteostasis and deposition of unfolded amyloid protein.

Several genes involved in the RAAS, including those for the angiotensin-converting enzyme, angiotensin and angiotensin receptor, have been associated with essential hypertension by candidate gene analysis [136–138]. Some studies of patients with essential hypertension have identified RAAS gene variants that are related to increased risk of AF [42, 79, 139]. Polymorphisms in this system have also been linked to stroke. However, the RAAS system is involved in multiple pathologies and attributing some variants to certain disease states might be challenging in the setting of so many confounders. Nevertheless, the possibility that some variants contribute to multiple pathologies through pleiotropy seems plausible, as AF, obesity, and hypertension are highly intertwined conditions and such pleiotropy has been well documented in other settings (Fig. 1).

Other clinical risk factors for AF that have been associated with common genetic variants include obesity and the metabolic syndrome. Obesity increases the risk of atrial fibrillation by almost 50 % [140]. In a GWAS meta-analysis of waist–hip ratio, *TBX15* was associated with increased waist–hip ratio. Unlike the other SNP hits in this study, *TBX15* did not differ by gender [141]. Candidate gene studies have shown that *CAV1* polymorphisms, resulting in decreased expression of the gene, are associated with insulin resistance and the metabolic syndrome in Hispanic and Caucasian cohorts [142, 143].

Conclusions

Genomic analyses have provided important insights into the pathogenesis of AF, though much still remains unknown.

While GWAS have identified common genetic variants associated with AF, identification of the causative genetic variants and their functional mechanisms remains challenging. For example, the top replicated AF-associated locus on chromosome 4q25 is in an intergenic region, but the functional variants and the direct mechanisms by which these variants increase AF susceptibility is still poorly understood. The nearest gene, *PITX2*, is a biologically attractive candidate causative gene, as it directs asymmetric morphogenesis of the heart, is involved in the formation of pulmonary veins, and represses a left atrial sinus node program that might contribute to pulmonary vein triggers. However, the connections between the AF-associated SNPs and gene expression or function of *PITX2* remain elusive. Transcriptional studies in atrial tissue have made connections between SNPs and genes in several other AF risk loci, including for *MYOZ1, CAV1, C9orf3*, *CAND2, GJA1*, and *TBX5*. Analyses also suggest that the delay in AF manifestation until later in life may be associated with impaired transcriptional responses to cellular stress. Future GWAS of AF with much larger sample sizes will undoubtedly increase the number of susceptibility loci that are significantly associated with AF risk. Further studies are needed to determine functional mechanisms by which these genetic loci contribute to AF risk, stroke, and/or other common co-morbid conditions. Understanding these mechanisms will set the stage for the development of new therapeutic agents that target the causative genes.

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Compliance with Ethical Standards

Conflict of Interest Alejandra Gutierrez and Mina K. Chung declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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