Recent advances in the genetics of atrial fibrillation: from rare and common genetic variants to microRNA signaling

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Abstract

Besides traditional risk factors, atrial fibrillation (AF) also shares a strong genetic component. Here, we review the genetics of AF including monogenic forms of AF, heritability of AF, complex genetic risk of AF, and the role of microRNAs in AF pathophysiology. Thirty-two mutations (17 genes) have been reported to cause familial AF. Mutations in cardiac ion channel genes or their subunits alter electrical properties and thereby lead to AF. Recently, also non-ion channel gene mutations have been identified to cause familial AF. Twin and community-based studies suggested AF to be heritable also on the population level. The AF risk in the offspring of an affected first-degree relative ranged between 2- to 5-fold, depending on the age of onset. Thereby, the risk of AF increases gradually the earlier the youngest relative of an AF patient developed the arrhythmia. African Americans bear a lesser risk of AF compared to individuals of European ancestry. Their risk rises with increasing European admixture. Genome wide association studies have revealed loci on chromosomes 4q25, 16q21 and 1q21 conferring risk of AF. Very recently, another consortial effort has identified a novel locus on chromosome 1, intronic to *IL6R*. *IL6R* encodes the α subunit of the interleukin 6 receptor. MicroRNAs were shown to regulate gene expression, and are increasingly reported to modify AF. A hallmark of AF pathophysiology is electrical and structural remodeling. MicroRNAs are involved in this process by regulating gene expression of cardiac ion channels, calcium handling proteins, transcription factors, and extracellular matrix related proteins.

Introduction

Genetic factors have been demonstrated to play a role in both rare and common diseases and conditions. However, the extent to which such genetic alterations contribute to disease pathophysiology varies. Besides syndromatic forms explained by a mutation, often common forms of the disease are known, where genetic variants are considered one fraction among several other risk factors. To this end, atrial fibrillation (AF) does not constitute an exception. It is the most common human arrhythmia, and affects millions of patients in Europe and worldwide.[1] Risk factors predisposing to AF have been identified and characterized for decades. Among them, the most important factors are age, sex, hypertension, valvular diseases and heart failure.[1-3] It was only in the last few years that also a heritable component of AF has been described independently of the established risk factors.[4,5] Subsequently, both monogenic forms of AF affecting few patients, and common forms with a lower genetic burden but affecting many people have been identified and the underlying genetic variation has been elucidated. Many findings in the field of genetics of AF have extensively been reviewed elsewhere.[6-11] However, thanks to many technological and methodological improvements, the field is moving fast forward. With this review article we intend to summarize the most recent findings regarding the genetics of AF, and put them into the context of existing data.

Recent findings for atrial fibrillation as a monogenic disorder

To date, 32 mutations in 17 different genes have been reported to cause AF (Table 1). The mutations cause AF either as an isolated arrhythmia, or in the context of complex arrhythmia syndromes like long QT syndrome, Brugada syndrome, or familial cardiomyopathies. Usually, mutations are rare, but result in strong effects and show a clear phenotype.[8] In selected cardiomyopathies AF may be a specific marker before structural changes or functional impairment become evident. Along this line incident paroxysmal AF in the context of prolonged PR interval and low amplitude P waves may be an indicator for laminopathy.[12,13]

Most of the mutations described for AF are located in genes encoding cardiac ion channels, and lead to altered electrical properties by causing either gain-of-function or loss-of-function effects. Cardiac potassium channels carry outward currents by which they establish the resting membrane potential and regulate myocardial repolarization.[14] Cardiac sodium channels mediate inward currents resulting in the initial rapid depolarization of the cardiac action potential. The balance between inward and outward currents during the plateau phase of the action potential determines the action potential duration (APD) and refractoriness.[15] As a consequence, mutations in genes encoding cardiac ion channels or their subunits lead to altered electrical properties and result in APD shortening, conduction slowing, shortening of atrial refractoriness, ectopic activity, or early / delayed after-depolarization. All of these changes can provide an atrial arrhythmogenic substrate leading to or sustaining AF.

Three gain-of-function mutations (S140G, V141M, S209P) have been reported in the *KCNQ1* gene encoding the α subunit of the delayed-rectifier potassium channel Kv7.1 (I_{Ks}).[16-18] The consequence is an increase in

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Table 1. Mutations associated with monogenic forms of atrial fibrillation.

Gene	Protein	Mutation	Gain-/loss-of-function	Reference
KCNQ1	Delayed-rectifier potassium channel (I_{Ks}), α subunit	S140G	Gain-of-function	16
KCNQ1	Delayed-rectifier potassium channel (I_{Ks}), α subunit	V141M	Gain-of-function	17
KCNQ1	Delayed-rectifier potassium channel (I_{Ks}), α subunit	S209P	Gain-of-function	18
KCNE2	Delayed-rectifier potassium channel, β subunit (MiRP1)	R27C	Gain-of-function	20
KCNE2	Delayed-rectifier potassium channel, β subunit (MiRP1)	L65F	Gain-of-function	21
KCNE3	Delayed-rectifier potassium channel, β subunit (MiRP2)	V17M	Gain-of-function	22
KCNJ2	Inward-rectifier potassium channel (I_{K1})	V93I	Gain-of-function	24
KCNA5	Ultrarapid delayed-rectifier channel (I_{Kur})	E375X	Loss-of-function	26
KCNA5	Ultrarapid delayed-rectifier channel (I_{Kur})	T527M	Loss-of-function	27
KCNA5	Ultrarapid delayed-rectifier channel (I_{Kur})	A576V	Loss-of-function	27
KCNA5	Ultrarapid delayed-rectifier channel (I_{Kur})	E610K	Loss-of-function	27
KCNH2	Rapid delayed-rectifier channel (I_{Kr})	N588K	Gain-of-function	25
SCN5A	Sodium channel, voltage-gated, type V, α subunit	D1275N	Loss-of-function	29
SCN5A	Sodium channel, voltage-gated, type V, α subunit	N1986K	Loss-of-function	30
SCN5A	Sodium channel, voltage-gated, type V, α subunit	M1875T	Gain-of-function	31
SCN5A	Sodium channel, voltage-gated, type V, α subunit	Y1795C	Gain-of-function	32
SCN1B	Sodium channel, voltage-gated, type I, β	R85H	Loss-of-function	34
SCN1B	Sodium channel, voltage-gated, type I, β	D153N	Loss-of-function	34
SCN2B	Sodium channel, voltage-gated, type II, β	R28Q	Loss-of-function	34
SCN2B	Sodium channel, voltage-gated, type II, β	R28W	Loss-of-function	34
SCN3B	Sodium channel, voltage-gated, type III, β	A130V	Loss-of-function	33
RYR2	Ryanodine receptor 2	p.Asn57-Gly91	Gain-of-function	35
NUP155	Nucleoporin	R391H	Loss-of-function	36
GJA5	Connexin-40	P88S	Loss-of-function	37
GJA5	Connexin-40	M163V	Loss-of-function	37
GJA5	Connexin-40	G38D	Loss-of-function	37
GJA5	Connexin-40	A96S	Loss-of-function	37
GJA1	Connexin-43	c.932delC	Loss-of-function	38
NPPA	Atrial natriuretic peptide	c.456-457delAA	Gain-of-function	39
TBX5	T-box transcription factor 5	p.Gly125Arg	Gain-of-function	40
GATA4	Gata binding protein 4	S70T	Loss-of-function	41
GATA4	Gata binding protein 4	S160T	Loss-of-function	41

current density and reduction in channel inactivation.[16,19] The genes *KCNE2*, *KCNE3* and *KCNE5* encode β subunits of potassium channels. Gain-of-function mutations in these genes (R27C (*KCNE2*), V17M (*KCNE3*), and L65F (*KCNE5*), respectively) have been described in AF patients, too.[20-22] The proarrhythmogenic effects of these mutations were ascribed to altered channel subunit assembly, a phenomenon that has been shown to affect potassium currents.[23] Xia *et al.* demonstrated the gain-of-function mutation V93I in *KCNJ2* that encodes the inward-rectifier potassium channel Kir2.1 (I_{K1}).[24] Hong and colleagues identified a family with short QT syndrome and a high incidence of paroxysmal AF due to a mutation in the *KCNH2* gene (N588K) that encodes the rapid delayed-rectifier potassium channel (I_{Kr}).[25] On the other hand, several loss-of-function mutations in the *KCNA5* gene (E375X, T527M, A576V, E610K) are associated with AF.[26,27] *KCNA5* encodes the ultrarapid delayed-rectifier channel Kv1.5 (I_{Kur}). Olsen *et al.* described a mutation of the *ABCC9* gene (T1547I) that encodes an ATP-binding regulatory subunit of K_{ATP} channel and leads to paroxysmal episodes of AF during physical activity.[28]

Interestingly, both loss-of-function mutations (D1275N, N1986K) as well as gain-of-function mutations (M1875T, Y1795C) in the *SCN5A* gene have been shown to be associated with AF.[29-32] *SCN5A* encodes the pore-forming α subunit of the voltage-gated cardiac sodium channel. Additionally, loss-of-function mutations in the β subunit genes *SCN1B* (R85H, D153N), *SCN2B* (R28Q, R28W) and *SCN3B* (A130V) were reported in AF patients.[33,34] However, in genes contributing to sodium channel formation, a clear genotype-phenotype correlation cannot be established in all mutation carriers. One explanation may be the fact that such mutations are not only linked to AF, but also to other arrhythmias including Brugada syndrome, long QT syndrome 3, idiopathic ventricular fibrillation or congenital sick sinus syndrome, affecting atrial and ventricular electrical properties as well as the cardiac conduction system on various levels. The result is a complex and overlapping pattern of phenotypes displayed across patients with sodium channel mutations.[8]

The ryanodine receptor gene *RyR2* encodes a calcium channel in the sarcoplasmatic reticulum that plays an important role in cardiac excitation-contraction-coupling.[15] In two families, a large deletion in *RyR2* was identified in AF-affected family members.[35]

Furthermore, mutations in genes coding for proteins other than ion channels have been reported in patients suffering from heritable AF. Zhang *et al.* reported a mutation in the nucleoporin gene *NUP155* (R391H).[36] Nucleoporins are components of the nuclear

pore complexes, and are involved in nucleus-cytoplasm interaction. However, their role in AF pathogenesis remains unclear. Gollob and co-workers demonstrated that mutations in the *GJA5* and *GJA1* genes are associated with lone AF. These genes encode the gap junction proteins connexin-40 and connexin-43.[37,38] The mutations in the gap junction genes may lead to impaired atrial electrical conduction properties promoting re-entrant circuits in AF. A mutation in the *NPPA* gene, encoding the atrial natriuretic peptide, was reported to be associated with AF creating an AF substrate by APD shortening.[39] Mutations in transcription factors like *TBX5* (p.Gly125Arg)[40] and *GATA4* (S70T, S160T)[41] are associated with AF presumably due to abnormal cardiac development.

Recent findings for atrial fibrillation as a heritable disease

A milestone on the way to identifying AF as a common disease with a complex genetic background was the understanding that the arrhythmia shares a heritable component. Initially, an accumulation of AF within a family was noted by Louis Wolff as early as 1940.[42] It then needed decades until the relation between AF onset and the presence of the arrhythmia in family members was described more formally. When studying the offspring of participants of the original cohort of the Framingham Heart Study, the investigators found that the odds of being affected by AF was almost 2-fold, when one parent had AF as well (odds ratio (OR) 1.85 (95% confidence interval (CI) 1.12-3.06, P = 0.02).[4] In a similar investigation in Iceland, the risk was 1.77 fold higher (95% CI 1.67.1.88, P < 0.001), when a first degree relative presented with the arrhythmia, too.[5] In both studies, the age of onset of AF in the relative played an important role: when the relatives themselves had developed AF at an earlier age (< 75 years, and < 60 years, respectively), the risk for the offspring increased in both investigations, and reached more than 3-fold in the Framingham Heart Study, and almost 5-fold in Iceland, respectively.[4,5] Heritability is a measure to quantify how much of the phenotypic variability of a disease or another phenotype is due to genetic factors. Heritability estimates can classically be derived from twin studies or population genetics. The higher the heritability is, the more important genetic factors are in explaining the condition. Twin studies in Denmark finally estimated a 62% heritability of AF.[43] Also, concordance rates for affection by AF was higher in mono- compared with dizygotic twins.[43]

Most recently, a number of new studies enriched the knowledge about AF heritability. Investigators from the Framingham Heart Study extended the insight into the relation between the incidence of AF and the presence of the arrhythmia in relatives.[44] Most importantly, the authors demonstrated an inverse relation between the risk of AF and the age at AF onset in the family members (Figure 1). Whereas the risk of AF was 1 if the affected relative developed the disease at age 90, the risk linearly increased to around 3-fold when the family member developed AF at young age. Numerically, the authors found a Hazard ratio of 1.32 (95% CI 1.12-1.56, P < 0.001) per decade of age less than at the age of AF onset. Of relevance was also the number of affected individuals per family; each additional affected increased the risk for the relative by 1.24 fold. All associations with AF risk remained consistent also after multivariable adjustment for numerous risk factors that have previously been shown to predispose to AF. However, the information of a positive family history itself did only subtly improve the ability to actually improve AF risk prediction.[44]

Another recent, and highly important investigation extended the information about the heritability of AF to African Americans; so far most studies were restricted to individuals of European descent.[45] It has repeatedly been demonstrated that African Americans are presenting with a markedly lower risk for AF,[1,46] whereas their prevalence of conventional risk factors like hypertension, diabetes or heart failure is higher than in patients of European descent.[47,48] Now, using data from the Atherosclerosis Risk in Communities (ARIC) Study, and the Cardiovascular Health Study (CHS), where both participants of African American and European descent were included, the investigators were able to show that one part of the racial differences can be explained by heritable, genetic factors (Figure 2).[45] These findings were possible, since the wealth of genetic information provided by genome wide genotyping arrays allows determining the ancestry information of the investigated DNA. Consistently across the independent studies, and after multivariable adjustment, for every 10% of European admixture to the African American gene pool, the risk of AF increased with a hazard ratio of 1.17 (95% CI 1.07-1.29, P = 0.001).[45]

Recent findings for atrial fibrillation as a complex genetic disorder

Based on the findings regarding AF as a heritable disorder on a population level, many attempts have been undertaken to elucidate the genetic causes underlying the heritability. Initially, case-control studies were designed to perform candidate-gene based genetic association studies. Many of these studies suggested positive associations of common genetic variants, mostly single nucleotide polymorphisms (SNPs), with AF. However, following a systematic replication study of such SNPs, only few results withstood showing significant replication.[49,50] Whereas these early studies constitute valuable contributions demonstrating the feasibility of identifying variants associated with AF, there are several reasons for the subsequent non-replication. These reasons include

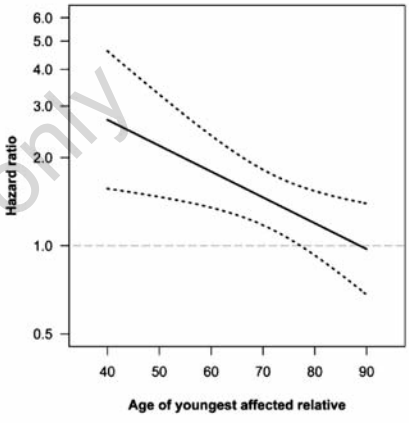

Figure 1. The figure displays the risk of developing atrial fibrillation (AF) depending on the age of AF onset in the youngest affected relative. The risk is highest, if the relative developed AF in the age of 40-50. With advancing age, the risk decreases. The hazard ratio for AF is plotted on the y-axis with a logarithmic scale. Dashed lines represent 95% confidence intervals. *Figure modified from Lubitz et al.[44], with permission.*

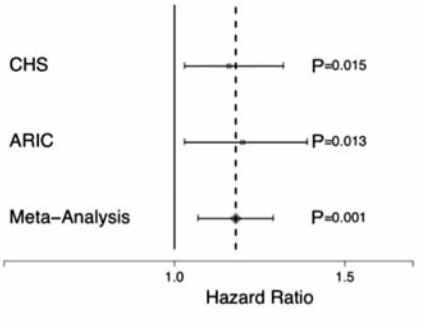

Figure 2. The Forrest plot displays the increased risk of atrial fibrillation (AF) in African Americans, depending on the European admixture. The meta-analysis showed a 17% risk increase per every 10% additional share of European descent, after multivariable adjustment. *Figure modified from Marcus et al.[45], with permission.*

commonly small sample size of the early studies, the low probability of randomly and still correctly selecting one single SNP from among millions of options, and the over-estimation of the actual risk conferred by an individual common genetic signal.[51-53] A systematic analysis of genetic studies of complex traits suggested that the median OR conferred by a single associated SNP is only 1.33 (25th, 75th percentile, 1.20, 1.61).[54]

Finally, the era of genome wide association studies (GWAS) assumed the recommendations of sufficient sample size and independent replication. So far, GWAS on AF have led to the discovery of three distinct chromosomal loci that confer risk of AF. First in 2007, a GWAS in individuals of European descent identified two SNPs at the chromosomal locus 4q25: rs2200733, OR 1.72 (95% CI 1.59-1.86, P = 3.3×10^{-41}), and rs10033464, OR 1.39 (95% CI 1.29-1.53, P = 6.93×10^{-11}).[55] Following the report, particularly rs2200733 has been widely replicated by various studies,[11,56-58] also involving participants of Han Chinese descent.[55,59] The signal also remained the strongest in all subsequent GWAS.[60-62] A follow up study aimed to more precisely define the genetic architecture at the 4q25 locus, and succeeded to identify two independent signals represented by the SNPs rs17570669 and rs3853445.[63] The Consortium for Heart and Aging Research in Genomic Epidemiology (CHARGE) then performed a second GWAS in a large, community-based sample of European descent, and succeeded to identify a second genome wide association signal. SNP rs2106261 is located on chromosome 16q21, and yielded a 1.25 fold increased risk of AF (95% CI 1.19-1.33, P = 1.8×10^{-15}).[60] Simultaneously, a second consortium identified the same locus [rs7193343, OR 1.21 (95% CI 1.14-1.29, P = 1.4×10^{-10})].[62] Both variants are in close proximity and are strongly linked to each other (linkage disequilibrium $r^2 = 0.78$ based on the HapMap 1000 genomes panel).[64] A third GWAS signal was found in AF patients with an early onset of the arrhythmia. The signal resides on chromosome 1q21, and the most significantly associated SNP is rs13376333 [OR 1.52 (95% CI 1.40-1.64, P = 1.82×10^{-21})].[61]

Regarding the pathophysiological mechanisms underlying the detected association loci, so far no unequivocal pathway has been demonstrated. The closest gene at the 4q25 locus is *PITX2* or the paired-like homeodomain 2 gene.[55] At the 16q21 locus, the most significant SNPs from both studies are intronic to the gene *ZFHX3*, encoding the zink finger homeobox 3,[60,62] while the signal on chromosome 1 is intronic to the gene *KCNN3* encoding the calcium-activated, small conductance potassium channel SK3 or KCa2.2.[61] All three genes are appealing candidates for involvement in AF pathophysiology, and the so far results regarding genetic and functional follow up studies have been reviewed in detail elsewhere.[6,9-11] Suggestive candidate genes have also been detected by two independent GWAS for the PR interval.[65,66] The PR interval can be quantitatively measured on standard electrocardiogram recordings, is a measure for atrio-ventricular conduction, and prolongation has been shown to be associated with AF and predict AF risk.[67-69] Loci that showed genome wide association with the PR interval have been also been tested for association with AF. Six loci tentatively involving variants in or around the genes *SCN5A, SCN10A, NKX2.5, CAV1 / CAV2, SOX5*, and *TBX5* were as well susceptibility loci for AF.[65,66]

Most recently, the list of associated loci was extended by another large consortial effort. The so called Candidate Gene Association Resource (CARe) of the United States National Heart Lung and Blood Institute encompassed individuals from ARIC, CHS, and the Framingham Heart Study.[70] Further samples were contributed by the German Competence Network on Atrial Fibrillation (AFNET). Genotyping was performed using the customary Illumina IBC SNP array. The SNPs on this array contained almost 50,000 variants that were specifically selected to represent the genetic variation in genes putatively relevant to cardiovascular diseases and conditions.[71] One main result of the study was the detection of rs4845625, a SNP intronic to the gene *IL6R*, which encodes the interleukin 6 receptor.[72] In the discovery cohorts, the variant decreased the risk of incident AF by 0.90 (95% CI 0.85-0.95, P = 0.00048). In the independent replication step in individuals with prevalent AF, the SNP remained significantly associated with an OR of 0.71 (95% CI 0.57-0.89, P = 0.003). A second important result of the investigation was the first time description of the new *IL6R* variants and the initial signal on chromosome 4q25 (*PITX2*) in African American study participants.[72] Whereas the *IL6R* SNP did not reach significance [relative risk 0.86 (95% CI 0.72-1.03, P = 0.09)], the *PITX2* SNPs previously reported for individuals of European descent, rs2200733, showed consistent strong association also in this ethnic group [relative risk 0.73 (95% CI 0.60-0.89, P = 0.0018)]. The most significant finding in African Americans at this locus was for SNP rs4611994 (HR, 1.40; 95% CI, 1.16-1.69; P = 0.0005), a marker in perfect linkage disequilibrium with rs2200733 ($r^2 = 1$).[72]

The association between the SNP in *IL6R* and AF did arise from a large, multi-national effort, and included an independent replication stage. However, possibly due to a remaining lack in statistical power, the association failed to reach the threshold of genome wide significance, which is commonly considered to reside at a P of 5×10^{-8}.[53] Until this goal will have been met in possibly upcoming analyses with higher numbers of participants, a certain lack in credibility of the association will remain. However, *IL6R* is an intriguing candidate, since other data are available supporting the relevance of the interleukin 6 receptor in the pathophysiology of AF. Particularly, it has been shown that variation in *IL6R* results in altered levels of the interleukin 6 receptor.[73] This relation has been demonstrated for the SNP rs8192284, which is in modest linkage disequilibrium with the top-SNP in the study described here ($r^2 = 0.5$).[64] Interleukin 6 itself has as well been reported to play a role in AF pathophysiology,[74,75] and genetic variation in the encoding gene *IL6* was suggested to modify the relation.[76] However, an independent replication of the finding has yet to be presented. In general, inflammation has repeatedly been suspected to constitute one pathophysiologic pathway in the development of AF.[74,77-79]

Several other recent studies aimed at contributing to the genetics of AF. Part of these studies was designed to add follow up or additional information regarding the GWAS signals reported so far. One study in patients who underwent coronary artery bypass graft surgery investigated the occurrence of post-operative AF conditional on SNPs at the 4q25 locus, and merit particular emphasis as it investigated a large cohort of close to 1200 patients.[80] More than one third of patients developed AF postoperatively, and both SNPs initially identified at the 4q25 locus (rs2200733 and rs10033464) were significantly associated with the incidence of AF after multivariable adjustment. A second analysis in the same cohort tried to link the two SNPs' genotypes as well to a long-term risk of AF following cardiac surgery, and again showed significant results for rs2200733, but slightly failed to reach significance for rs10033464.[80] In both analyses, the hazard ratios for AF were somewhat smaller compared to the initial description of the association in the Icelandic community as well as compared to a recent large-scale meta-analysis.[11,55] In the present study, the hazard ratio was 1.41 for direct postoperative AF, and 1.32 for long-term incidence of AF following surgery.[80] In contrast, the meta-analysis of community-based AF samples reached an OR of 1.68.[11] One might interpret these circumstances in a sense that the genetic contribution of markers at the 4q25 locus is of lesser importance when a strong non-genetic trigger for AF, like open-heart surgery, is present.

Two other recent studies dealt with rs2200733 and AF. Yet, both sample sizes were small so that the reliability of their results has to be questioned. One study in 196 patients with lone AF and 176 controls failed to replicate the association.[81] However, another study involved 219 cases with paroxysmal lone AF, who had episodes of sinus rhythm allowing for measurements of the PR interval.[82] The

authors succeeded to demonstrate, that the rare allele of rs2200733 is associated significantly with prolonged PR intervals. While the authors noted that the PR interval could be considered a valuable intermediate phenotype for lone AF, it has to be highlighted that despite the limited statistical power of the study no independent replication was attempted. Two large-scale consortial GWAS meta-analyses, each involving several thousands of patients, did not suggest *PITX2* variants to be associated with the PR interval.[65,66]

The second GWAS signal for AF, described at chromosome 16q21 and supposedly involving the transcription factor *ZFHX3*, was the aim of a recent study in participants of Han Chinese descent.[83] The authors attempted a replication analysis in this ethnic group (650 AF cases, 1447 controls). Although both SNPs initially described by the two GWAS consortia were examined,[60,62] only rs2106261 replicated and after multivariable adjustment was associated with an OR of 1.29 (P = 0.001).[83] The association tended to be stronger, when only cases with lone AF were considered. The fact that the second SNP at the 16q21 locus did not replicate may shed some light on the potential differences in the genetic architecture between individuals of European *vs.* Han Chinese descent. The latest HapMap built based on the 1000 genomes data reveals an $r^2 = 0.789$ for Europeans, while the linkage disequilibrium in Han Chinese only has an $r^2 = 0.211$.[64] These differences could explain why both rs2106261 and rs7193343 reached genome wide significance in Europeans, but only rs2106261 was associated in the Chinese.

A last investigation that was triggered by the report of GWAS findings for AF screened the exonic regions of *KCNN3* in a cohort of just over 200 patients with lone AF and comparably many controls. While the authors did not identify any rare mutations, a SNP (rs1131820) showed significant association with AF.[84]

Finally, a number of recent studies conducted genetic analyses in patients with AF, and tried to identify or substantiate susceptibility loci that have not previously been identified by GWAS. One interesting example was conducted in and around the gene *GJA5* encoding the gap-junction protein connexin-40.[85] The authors were able to identify that a common polymorphism in the promotor region of the gene, rs10465885, showed highly significant association with the expression levels of *GJA5* (P < 0.0001). Subsequently, the group aimed at associating the SNP with AF. They genotyped cases and controls from the ARIC Study, and from the Massachusetts General Hospital (cases) and the Framingham Heart Study (controls), respectively, and in both instances independently found a significant association.[85] Overall, the study is an important example of an investigation, where – despite by far missing genome wide significance – pathophysiologically relevant results were substantiated by functional data.

One study in Chinese individuals of Uigur descent involving 303 AF cases and 328 controls suggested that rs1805127 in the gene *KCNE1* may be associated with an increased risk of AF;[86] the finding remained unreplicated. Other genes, encoding potassium channels *KCNJ2*, *KCNJ3*, and *KCNJ5*, were studied by a research group from Denmark.[87] All genes were sequenced for the protein coding sequence, and no mutations were detected. Two common polymorphisms in *KCNJ5*, though, were associated with AF in 187 patients with early onset of the arrhythmia, and a similar number of controls. Exploring a different potential pathway, the same group from Denmark included 158 AF patients and 188 AF-free controls and studied several polymorphisms in the genes *IL1A, IL1B, IL10, IL18,* and *TNF*, all of which are involved in the regulation of the immune system.[88] However, none of the associations turned out to be significant. A weak point of both studies has to be considered the very low sample size, which might have lead to false-negative non-associations.

A number of studies aimed at the renin-angiotensin-aldosterone system. A group of Chinese investigators genotyped a total of 620 hypertensive AF cases/controls for a variant in the gene encoding the aldosterone synthase, but found no significant relation.[89] The gene encoding angiotensinogen and three of its polymorphisms was the target of a small study in Turkish patients.[90] Comparing 100 AF cases with 100 controls, the authors claimed an association for two of the variants, commonly referred to as M235T and G-6A. In the same study, also the angiotensin converting enzyme insertion/deletion polymorphism (*ACE* I/D) was examined, and a statistically significant association was described.[90] All three results suffer from a very low sample size and are therefore questionable.

More informative due to the increased statistical power is a recent meta-analysis for the *ACE* I/D polymorphism, which included 18 case-control studies and a total of 7577 patients.[91,92] Such an analysis was much needed since the many different data sources describing findings regarding the *ACE* I/D polymorphism were highly conflicting. The main finding of the meta-analysis was that the overall power of the analysis was still insufficient to reach a convincing conclusion. No association was found when an additive or a dominant model of inheritance was assumed, but significance was reached assuming a recessive model.[91]

Considering relevant heterogeneity across the meta-analyzed studies, it appeared that the *ACE* I/D polymorphism might be of particular importance in hypertensive patients. The meta-analysis did not include the Turkish study mentioned above,[90] and also did not include a recent analysis in almost 3000 AF patients and over 5000 control patients recruited in Germany and the US.[49]

Both studies were not yet available when the meta-analysis was published, and thus the relevance of the *ACE* I/D polymorphism still has to await final clarification with respect to the involvement in AF development.

Recent findings for miRNAs and their involvement in the genetics of atrial fibrillation

So far in this review article, we described the variation (mutations and SNPs) in the genomic DNA that is involved in the heritability of AF. Besides DNA, also other regulating genetic and genomic elements are known to be involved in gene expression and modifications of the cell cycle. In particular, small RNA molecules, not coding for proteins, came into the focus of research. Such molecules are not inherited in a traditional, Mendelian fashion. However, their function has been recognized to play a major role modifying classical genetic pathways, and influencing phenotype severity. In 1993 Lee and colleagues described that the gene *lin-4* does not encode a protein but rather a pair of small RNAs involved in the regulation of C. elegans development.[93] Over the following years, other small, non-coding RNAs, now referred to as microRNAs (miRNAs), were described in other species including humans.[94]

In general, miRNAs are small (approximately 20-25 nucleotides) non-coding single-stranded RNA molecules that regulate post-transcriptional gene expression by binding to the 3'-UTR of their target genes. The result is an inhibition of translation, mRNA deadenylation or mRNA degradation.[95] It is estimated that more than 1000 distinct miRNAs are encoded in the human genome. Each miRNA can target several mRNAs; miRNAs thus establish a complex network of possible miRNA-mRNA interactions. Each cell type in each developmental stage displays a specific miRNA expression pattern that is genetically determined.[96] As a consequence, a cell- and time-specific miRNA microenvironment is established under healthy conditions, enabling miRNAs to play an important role in fine-tuning or *micromanaging* the output of the transcriptome.[96]

Regarding myocardial miRNA expression, interesting functions have been reported for miR-208, which acts as an on-off switch.[97] An important pathophysiologic process in heart disease is the re-expression of a foetal gene expression pattern. The adult, fast-contracting

α-myosin heavy chain (MHC) is downregulated, whereas the usually embryonic, slow-contracting β-MHC is upregulated in response to cardiac stress. The DNA encoding miR-208a is located in an intron of the α-*MHC* gene, and has been shown to play an important role regarding the isoform switch: downregulation of α-*MHC* consequently results in downregulation of miR-208a, and thus in a reduction of the repressive effects it usually has on β-*MHC* gene expression.[98]

Besides the cardiac- (miR-208a/b and miR-499) and muscle-specific miRNAs (miR-1 and miR-133), other miRNAs are expressed in adult myocardium. In healthy myocardium, their expression levels are relatively low but a marked increase has been described under pathologic conditions.[99] Depending on the pathological trigger, a specific subset of miRNAs is upregulated. Examples are miR-320 in myocardial infarction,[100] or miR-9/-195 in cardiac hypertrophy.[101,102]

Additionally, several microRNAs are involved in AF pathophysiology due to their regulatory actions in atrial electrical and structural remodelling. Yang and co-workers suggested an important role for miR-1 in the regulation of cardiac excitability.[103] The authors demonstrated that upregulation of miR-1 in patients with coronary artery disease caused repression of *GJA1* and *KCNJ2*, genes that code for connexin-43 and I_{K1}, respectively. This in turn resulted in conduction slowing and depolarization of the cell membrane. Such changes might imply a potential arrhythmogenic role for miR-1. In support of their hypothesis, the authors could show that knockdown of miR-1 in a rat model of myocardial infarction suppressed arrhythmogenesis.[103] However, another study suggested knockdown of miR-1 can also lead to conduction slowing because of reduced *KCND2* expression (via targeting the transcription factor Irx5).[104] Luo *et al.* revealed that miR-1 also acts as a post-transcriptional repressor of *KCNE1*, a subunit of the potassium channel responsible for the slow delayed rectifier current I_{Ks}.[105] Terentyev *et al.* performed experiments on rat ventricular cardiomyocytes, and were able to show that miR-1 overexpression also leads to marked changes in calcium cycling and excitation-contraction coupling.[106] B56α, a regulatory subunit of protein phosphatase 2A (*PP2A*) was identified as a miR-1 target. MiR-1 overexpression led to increased phosphorylation of the ryanodine receptors, resulting in an elevated diastolic calcium leak from the sarcoplasmic reticulum (SR) and reduced SR calcium content. Girmatsion *et al.* examined atrial tissue and found a significant increase in *KCNJ2* expression as well as an increase in Kir2.1 protein and I_{K1} density when miR-1 was significantly downregulated in atrial tissue.[107] Changes in inward rectifier potassium currents play an important role in AF maintenance by APD shortening and hyperpolarizing atrial cardiomyocytes. Thereby the voltage-dependent inactivation of I_{Na} is reduced.[108] In conclusion, miR-1 downregulation or upregulation can result in cardiac electrical remodelling leading to increased AF vulnerability. The changes support the idea of miR-1 as a fine-tuner of cardiac electrical properties.

Also miR-26 was mentioned as a player in AF pathophysiology.[109] MiR-26 is significantly downregulated in AF leading to upregulation of its target gene *KCNJ2*. Regarding the underlying mechanism, Luo *et al.* revealed that the enhanced activity of the transcription factor NFAT seen in AF, causes direct repression of miR-26 with a consecutive increase of the I_{K1} current.[109]

Lu and co-workers performed experiments on a canine atrial tachypacing model and illustrated that miR-328 might play an important role in AF by repressing *CACNA1C* and *CACNB1*. Both genes encode the cardiac calcium channel $I_{Ca,L}$.[110] Following tachypacing, the authors measured a significant increase of miR-328 expression in right atrial tissue and confirmed their results in human atrial tissue. Overexpression of miR-328 in dogs and mice displayed a similar phenotype with reduced $I_{Ca,L}$, atrial APD shortening and enhanced AF vulnerability.[110]

MiR-133 is a muscle-specific miRNA that regulates expression of several ion channel genes and is therefore involved in AF pathophysiology. It affects the transient outward potassium current $I_{to,f}$,[111] and the slow delayed-rectifier potassium current I_{Ks}[105] by targeting *KCNIP2* and *KCNQ1* respectively. Furthermore, miR-133 plays an important role in atrial structural remodelling. Shan *et al.* demonstrated in a canine tachypacing model and in canine atrial fibroblasts that treatment with nicotine stimulates collagen synthesis and atrial fibrosis.[112] As the underlying mechanism the investigators found a downregulation of miR-133 and miR-590, and an upregulation of profibrotic TGF-β1 and TGF-β1 receptor type II. Interestingly, TGF-β1 and TGF-β1 receptor type II could be identified as targets of miR-133 and miR-590. Transfection of atrial fibroblasts with miR-133 and miR-590 showed similar expression changes; miRNA antagonism abolished these effects.[112] Duisters *et al.* reported that miR-133 and miR-30 target the profibrotic connective tissue growth factor (CTGF).[113] In cardiac hypertrophy, these miRNAs are downregulated, whereas CTGF is upregulated, resulting in increased fibrosis. The results could be confirmed *in vitro* by knockdown and overexpression experiments.[113]

Thum and colleagues performed experiments in a mouse model of cardiac hypertrophy and could show that miR-21 levels are significantly increased in cardiac fibroblasts in the failing heart.[114] The upregulated miR-21 causes an increase in ERK-MAP kinase activity by repressing its target gene sprouty homologue 1 (*Spry1*). As a consequence, fibroblast survival, growth factor secretion, the extent of cardiac fibrosis, and cardiac hypertrophy are enhanced. *In vivo*, antagonism of miR-21 reduced cardiac fibrosis and improved cardiac function. Another study on miR-21 in a murine model of myocardial infarction revealed that miR-21 is downregulated in the infarcted tissue. One consequence is a reduction of the miR-21 repressive effect on its target phosphatase and tensin homologue in fibroblasts. The result is a significant upregulation of matrix metalloproteinase 2.[115] The study results might establish a potential role of miR-21 as a mediator of structural remodelling in heart disease. However, the results are still under debate.[116,117]

In a mouse model of myocardial infarction, van Rooij *et al.* observed a significant downregulation of miR-29 in the border zone of the infarction.[118] The authors showed that downregulation of miR-29 *in vitro* and *in vivo* induces cardiac fibrogenesis by de-repressing gene expression of collagens, fibrillin and elastin. In contrast, forced overexpression of miR-29 in fibroblasts reduces profibrotic gene expression. In a canine tachypacing model, Dawson *et al.* reported a marked downregulation of miR-29b in atrial tissue, associated with upregulation of profibrotic gene expression, cardiac fibrosis and vulnerability to AF. The authors could also confirm the causal action of miR-29b by performing *in vitro* manipulation experiments.[119]

Conclusions

Since the time that a genetic contribution to AF has first been substantiated, numerous studies contributed important findings towards a more detailed picture. Genetic factors have now been shown to play an important role in various aspects of the arrhythmia. While rare mutations cause familial forms of AF only in a small fraction of patients with the disease, the functional assessment of the mutation effects contributed strongly to our understanding of AF pathophysiology. On the other end of the spectrum, common SNPs affect a large number of individuals, but the effect sizes conferred by a single common variant are often minuscule. Despite the fact that large consortia with tens of thousands of included participants conducted GWAS, only relatively few susceptibility loci for AF have been identified. The common variants that so far have been linked to AF explain only a fraction of the entire heritability of the arrhyth-

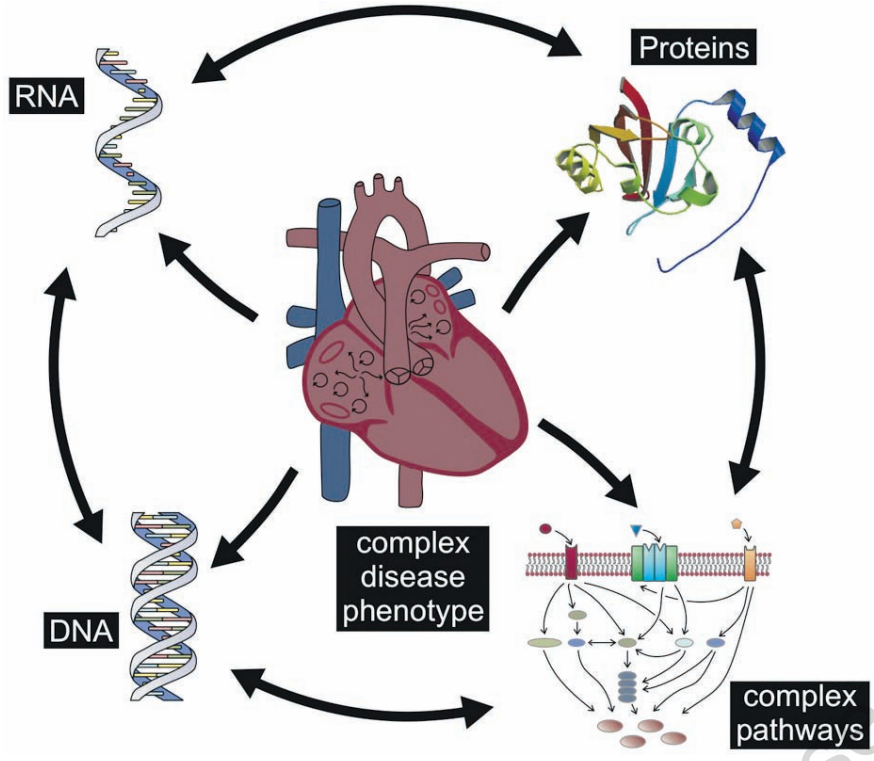

Figure 3. Schematic illustration of atrial fibrillation (AF) pathophysiology. A complex phenotype like AF cannot be explained by a single causal mechanism but rather by a complex interaction of different factors. Transcription, translation, protein subunit assembly or conformational changes as well as complex signaling pathways are involved in AF. These mechanisms interact with each other leading to AF development and are in turn influenced by AF.

mia.[6] The future will have to initiate the search for the missing pieces to explain the genetic background of AF. One fraction is likely to be detected in low frequency variants. These variants are supposed to be more frequent than mutations, but less frequent than SNPs. In turn, their effect size is expected to be higher than that of the so far detected SNPs. Another share of the genetic background of AF might hide in other DNA and RNA molecules like miRNAs. The continued investigation of this emerging field is crucial. Overall, the genetic background of AF is diverse and the future will show many layers that interact in a multitude of ways. Figure 3 depicts a possible integration of various pathways: AF is a complex phenotype in the center. It is influenced, caused and modified by many surrounding systems. DNA and the associated mutations and SNPs build one column; RNA molecules like miRNAs build another. Other fields include protein interactions and other complex pathways that were not part of this review article. All fields interact both with AF, but also among each other. Taken together, a system of mechanisms finally makes up the complex picture behind AF pathophysiology. Despite further studies on each field represented in the figure, also a comprehensive, systems biology approach is warranted to elucidate all aspects of AF.

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