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FAMILIAL ATRIAL FIBRILLATION AS A POLYGENIC DISEASE WITH STRUCTURAL CARDIAC ABNORMALITIES: ASSESSMENT OF GENETIC RISK AND POSSIBILITIES FOR GENE THERAPY

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The prevalence of familial atrial fibrillation (AF) in the general population and in the structure of AF is considered, and genetic predictors of AF and pathogenetic mechanisms of atrial remodeling are analyzed. The assessment of the genetic risk of AF occurrence, the prediction of its outcomes and the effectiveness of AF therapy, as well as the prospects for AF gene therapy are discussed.

Key words: antiarrhythmic therapy; genetic testing; catheter ablation; atrial cardiomyopathy; atrial fibrillation

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Atrial fibrillation (AF) is the most common arrhythmia affecting up to 1% of the population worldwide [1, 2]. The prevalence of AF increases exponentially with age and can reach the mark of 8% in the elderly population [3]. Epidemiologic studies have confirmed the essential importance of the genetic aspect in the pathophysiology of AF [4]. Currently, more than 160 genes have been found to be associated with AF [5]. Some have been identified using classical linkage studies, but most rely on functional or genome-wide association studies [6]. Genome-wide association studies (GWAS) in individuals with documented familial AF have identified common single nucleotide polymorphisms associated with AF [7, 8].

Depending on the underlying cause of AF, there are differentiated: AF caused by external risk factors, the so-called acquired AF; congenital AF and genetic (familial) AF [2]. Acquired AF is associated with the effects of aging as well as risk factors such as arterial hypertension, diabetes mellitus, obesity, coronary heart disease, and chronic kidney disease. Approximately 5% of patients with congenital heart disease develop AF due to a combination of embryogenesis and peri- and postoperative factors related to the correction of the heart defect [9, 10]. Congenital AF is characterized by the onset of AF at a younger age and a relatively rapid transformation of paroxysmal AF into persistent AF [3, 11]. In about 15% of patients with congenital AF, it is familial, suggesting a genetic predisposition [12]. The interaction between genetic predictors and acquired risk factors for AF is also important [13].

EPIDEMIOLOGY OF FAMILY FORM OF AF

The inheritability of AF has been extensively investigated since the first report of family form of AF in 1936 [11]. This is due to the high prevalence of isolated AF and differences in its incidence according to gender and ethnic groups [1, 7]. The frequency of familial form of AF is unknown, but recent studies suggest that up to 30% of patients with isolated AF (i.e., without known cardiac pathology or risk factors) have a history of the disease in their family [6, 11]. L.C.Weng, et al. [8], based on the study of common genetic variants of AF, showed that the inheritance of AF in people of European descent is about 22% of all cases of AF. In the Framingham Heart Study, having a family history of AF was associated with a 40% increased risk of AF [2]. In the Mayo Clinic AF registry, 5% of all patients and 15% of patients with isolated AF had a family history of AF [14].

A population-based cohort study of patients with AF demonstrated significant familial incidence of AF and a high probability of heritability among patients with AF. According to Christopherson et al. [5], among 5000 Icelanders, first-degree relatives of patients with AF were 1.8 times more susceptible to the development of AF than in the general population, and in patients younger than 60 years of age, the relative risk of AF reached 4.67. In a study of Danish twins, the risk of developing AF was 12% for monozygotic twins and 22% for dizygotic twins [5]. It is found that more than 60% of the variance in AF is explained by genetic effects. The remaining heritability of AF can be explained by promoter variants, epigenetics, structural variants, and undiscovered genetic mechanisms [11]. Recommendations for the clinical use of genetic testing for familial AF are described in the Genetics Home Reference at https://medlineplus.gov/genetics/condition/familial-atrial-fibrillation [16].

L.Staerk et al. [1] showed that the incidence rate of familial AF was 3.48 in patients in whom first-degree rel-

atives were affected and 1.64 in those in whom second-degree relatives were affected. An increased risk has been identified, especially if there are multiple affected relatives and relatives with onset of AF at a young age. The ORBIT-AF registry showed that patients with familial AF had more symptoms than other variants of AF [15]. However, there were no differences between the two groups in terms of AF recurrence, hospitalization rates, complications, and overall mortality.

GWAS have identified more than 100 genetic loci associated with AF [17]. Most of them point to ion channels, transcription factors, and regulatory genes involved in the mechanisms leading to the development of AF (Table 1). The GWAS consensus implies that AF is both polygenic and pleiotropic in nature [6]. With the advent of whole genome and whole exome sequencing, both common and rare genetic variants of AF have been identified and linked to the pathogenetic basis of the familial form of AF [4].

Among the genes involved in the realization of various pathogenetic mechanisms of AF occurrence there are genes affecting potassium channels (*KCNA5, KCND3, KCNE1, KCNE2, KCNE3, KCNE4, KCNE5, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNN3, KCNQ1, ABCC9*), on sodium/potassium channels (*HCN4*), and on sodium channels (*SCN1B-4B, SCN5A, SCN10A*); genes involved in cellular calcium homeostasis (*RyR2, CACNB2, CACNA2D4*); genes involved in the development of fibrosis and remodeling of the extracellular matrix (*NPPA. MMP3, COMP, COL12A1, COL23A1, COL21A1, ANGPTL2, COLQ*); genes involved in cardiac morphogenesis (*GATA4, GATA5, GATA6, GREM2, NKX2-6*); genes involved in intercellular communication (*GJA1, GJA5*) and genes involved in nuclear structure (*LMNA, NUP155*).

COMMON GENETIC VARIANTS OF AF

Familial AF is very heterogeneous and may have autosomal dominant or recessive inheritance [4, 14]. A GWAS meta-analysis of more than 50 studies involving more than 65,000 patients with AF found a more than 3-fold increase in the number of loci associated with AF [17]. An association between earlier onset of AF and high genetic risk of AF variants has also been found [18].

It was shown that the most significantly associated with familial AF single-nucleotide polymorphism is in the noncoding region of chromosome 4q25 of the *PITX2* gene (paired homeodomain-2 gene) [19]. In addition, *PITX2* expression is significantly reduced in patients with AF, suggesting a link between loss of function in *PITX2* and AF [14]. In experiment, loss of *PITX2* function was found to be associated with sarcomere disruption, increased fibrosis, and a more than 4-fold increase in *HCN* passthrough gene expression [6, 19].

Another significant single nucleotide polymorphism identified using GWAS is the rs2106261 locus located on chromosome 16q22, intronic to the *ZFHX3* transcription factor gene [4]. The *ZFHX3* gene is expressed in the heart and is associated with myogenic and neuronal differentiation [8]. The association of AF with the *KCNN3* gene (locus located on chromosome 1q21), which encodes the calcium-activated potassium channel SK3 and is involved in atrial repolarization, has also been revealed [17]. Blocking these channels leads to antiarrhythmic effects by selectively prolonging the action potential (AP) in the atria [18].

In addition, two identified loci were located near genes that are targets for antiarrhythmic drugs, *SCN5A* and *KCNH2* [20]. The *SCN5A* gene, encoding Nav1.5 channel, is a target for sodium channel blockers, and the *KCNH2* gene, encoding Kv11.1 channel, is a target for drugs that inhibit potassium channels [13]. *KCNH2* gene variants associated with both loss-of-function and gain-of-function Kv11.1 channels are associated with frequent paroxysms of AF.

AF has also been reported to be associated with two common variants in the *RPL3L* gene on chromosome 16 and one variant in the *MYZAP* gene on chromosome 15 [21]. Another locus associated with AF was found on chromosome 10q22 and is located near the *SYNPO2L* and *MYOZ1* genes. The structural proteins encoded by these genes are expressed in both skeletal muscle and heart and are closely associated with the phenotype of atrial cardiomyopathy (ACM) [8]. I.E.Christophersen, et al. [5] identified 12 new loci of AF using GWAS involved in genes involved in structural remodeling of the heart. The most significant association was observed at locus 2q31, carrying seven highly correlated missense variants of the *TTN* (connectin, encodes the protein titin) gene, which is a strong candidate gene for AF that is involved in myocardial structural integrity and elasticity [22].

Transcription factors have been shown to play an important role in predisposition to familial AF [23]. They bind to specific DNA sequences in the promoter regions of genes and regulate their expression. Cardiac-specific transcription factors are involved in the regulation of gene expression (e.g., *GATA4, GATA6, MYH6, NKX2-5, PITX2*) involved in the formation of cardiac structures and the conduction system and are also associated with the risk of developing AF [17].

Given the complex polygenic origin of familial AF, which is important for disease outcomes and choice of therapy, 4 phenotypes of familial AF have been conventionally identified: Phenotype A (genes encoding various peptides and enzymes, e.g., *NPPA, PRKAG2*, angiotensin-converting enzyme - ACE); Phenotype B (various transcription factors; e.g., *PITX2, TBX5, ZHX3*); phenotype C (genes involved in the formation of structural components of the heart; e.g., *MYL4, TTN*) and phenotype D (genes encoding ion channel functions; e.g., *KCNQ1, SCN5A*).

RARE GENETIC VARIANTS OF AF

The first association between rare variants in the *KCNQ1* gene encoding the α-subunit of slow potassium current I_{Ks} and familial AF was found in 2003 [24]. β-subunits of potential-dependent potassium channels are encoded by *KCNE1-KCNE5* genes and carry rare variants associated with isolated and familial AF [12]. The functional effects of these variants are associated with an increase in current I_{Ks} and potential effects on transient sodium current (I_{to}) and fast potassium current (I_{Kr}).

A rare variant of the *KCNH2* gene, which encodes the α-subunit of the fast potassium current channel I_{Kr}, has been identified in a family with AF and shortened QT

interval syndrome, suggesting overlapping phenotypes [24]. The Kir2.1 inward rectifier channel mediates the abnormal I_{K1} potassium current involved in repolarization and is encoded by the *KCNJ2* gene. Functional analysis demonstrated enhanced channel function, suggesting a role for this gene in the initiation and/or maintenance of AF [12]. In a cohort of patients with AF, rare variants were also found in the *KCNJ8* gene encoding the Kir6.1 channel and in the *KCNJ2* gene encoding the α-subunit of the Kir3.4 channel [25].

Of particular interest is the *KCNA5* gene, which encodes an atrial-specific Kv1.5 channel involved in cardiac repolarization. I.E.Christophersen, et al. [5] identified various rare variants in the *KCNA5* gene in patients with early onset of isolated AF, both with loss of function and with gain of function of the Kv1.5 channel, which provides ultrafast potassium current (I_{Kur}), which increases susceptibility to AF.

AF has also been found to be associated with genes encoding potential-dependent sodium channels. For example, about 10 rare variants of the *SCN5A* gene have been identified in patients with early-onset AF, and most of them were previously diagnosed with prolonged QT interval syndrome [26]. Functional studies revealed abnormalities in both transient sodium current (I_{to}) and an increase in steady-state sodium current.

In addition, variants in four β-subunit sodium channels encoded by the *SCN1B-SCN4B* genes have been identified in patients with familial AF. Variants in these genes cause changes in the gating properties of sodium channels and attenuation of sodium current [25]. Ten rare missense variants of the *SCN10A* gene encoding the Nav1.8 sodium channel have also been found in patients with isolated AF. Functional studies revealed both gain and loss of Nav1.8 channel function, suggesting the involvement of *SCN10A* in the development of familial AF.

It should be noted that enhanced diastolic release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum into the cytoplasm via ryanodine receptor type 2 (RyR2) is one of the mechanisms of AF development [27]. Increased expression of *RYR2* gene in atria has been found in patients with paroxysmal AF [17]. It has been shown that microRNA (miRNA)-mediated posttranscriptional regulation of *RYR2* may be the main mechanism of AF development [23]. A full-exome study in families with early-onset AF revealed rare variants in the *CACNB2* and *CACNA2D4* genes, which encode L-type calcium channels with overlapping effects on Cav1.2, emphasizing the important role of these genes in predisposition to AF [28].

Recently, there is increasing evidence that structural genes are involved in the development of familial AF [13, 17, 21, 29]. An increased role of fibrosis and atrial ACM in the pathogenesis of AF has also been reported [4, 29]. These findings challenge the traditional view of AF as an electrical disease and allow for improved diagnosis and treatment of AF in the future [2, 4, 6, 10].

A homozygous variant, c.1172G> A in the *NUP155* gene was found to be segregated in family members with AF [17]. The *NUP155* gene encodes nucleoporin, which is a major component of nuclear pores involved in cytoplasmic transport. A variant of the *NPPA* gene has been identified in a family with autosomal dominant inheritance of AF [20]. *NPPA* encodes an atrial natriuretic peptide involved in the regulation of blood pressure. Rare variants in *MYH7, MYBPC3, MYL4* and *TTN* genes have been found to be associated with atrial ACM [30], which is characterized by altered sarcomeric architecture that contributes to re-entry and AF [22].

Intercellular gap junctions have been found to play an important role in the arrhythmogenesis of AF. For example, connexin-43 and connexin-40, encoded by the *GJA1* and *GJA5* genes, respectively, are gap junction proteins in the atrial myocardium [31]. An increased risk of AF with polymorphisms in the renin-angiotensin-aldosterone system (RAAS) genes encoding ACE inhibitor and angiotensinogen have also been reported [7, 32].

AF as a polygenic disease with a structural component is associated with different variants of genes encoding cytoskeletal proteins [4]. Thus, the most common variants of *MYH7* and *MYBPC3* genes are associated with hypertrophic ACM [33]. Arrhythmogenic right ventricular ACM has also been shown to be associated with variants in intercalated disc genes, and patients with this condition have an increased risk of AF and ventricular arrhythmias [6].

O.B.Vad et al. [34] identified rare loss-of-function variants in three different genes of dilated BMP (*DMD, PDLIM3, FKTN*) associated with early onset of AF, which is probably due to the development of atrial ACM. In addition, atrial ACM has been found to be associated with the *MYL4* gene (myosin-4 light chain gene), which is responsible for the electrical, contractile, and structural integrity of the atria [34]. A *MYL4* variant associated with a high risk of stroke has been identified in a patient with atrial ACM and recessive form of AF [22]. H.Cochet et al. [35] found a high degree of re-entry activity in the atrial fibrosis zone in patients with persistent AF. Drug blockade of RAAS has been found to reduce atrial fibrosis and duration of AF.

GWAS studies have identified genes associated with AF that are involved in various inherited arrhythmias, conduction diseases and cardiomyopathies [4]. This emphasizes the pleiotropy of these genes as well as the polygenic nature of AF. Overlap syndromes of AF with other hereditary arrhythmia phenotypes such as Brugada syndrome, prolonged and shortened QT interval syndromes have been identified [6]. Patients with congenital long QT syndrome have been shown to have a higher risk of early onset of AF than in the general population [26]. In patients with Brugada syndrome, the incidence of isolated AF ranges from 11% to 39%, being an indicator of poor prognosis [36].

Increased expression of *MYH6* and *MYH7* genes in atria was also found. The *MYH6* gene encoding the α-subunit of myosin heavy chain (α-MyHC) has been shown to be associated with AF and sinus node dysfunction, and the *MYH7* gene encoding the β-subunit of myosin (β-MyHC) has been shown to be associated with chronic AF [33]. β-MyHC is activated in heart failure and other cardiac diseases, whereas α-MyHC is suppressed, which confirms the role of MyHC isoforms in determining cardiac contractility. Another study identified a variant in the *PLEC* gene, which encodes structur-

al components of the cardiomyocyte, and was associated with a 55% increased risk of AF and 64% increased risk of sinus node dysfunction [21].

ELECTRICAL AND STRUCTURAL REMODELING OF ATRIA - THE PATHOGENETIC BASIS OF AF

The pathogenesis of AF is poorly understood, which to some extent complicates the development of effective treatment methods. Variants in genes encoding ion channels, signaling molecules, additional subunits, and gap junctions associated with AF have been shown to lead to the development of AF by different pathways [7, 30].

Atrial remodeling likely begins with electrical remodeling characterized by a reduction in atrial refractoriness, an increase in repolarization dispersion, and a slowing of conduction [30]. These changes occur because of abnormalities in AP currents caused by excessive $Ca2^{+}$ influx into cardiomyocytes and impairment of its subsequent homeostasis. Further, alterations in the $Ca2^{+}$ exchange cycle contribute to ectopic activity and diastolic $Ca2^{+}$ leakage from the sarcoplasmic reticulum via RyR2 receptors [27]. As a result, atrial re-entry circulation is stabilized and atrial vulnerability to AF is increased [13].

Due to atrial structural changes caused by variants in genes encoding myocardial cytoskeletal proteins, fibrosis and atrial ACM develop, which contribute to increased myocardial collagen volume and decreased intercellular gap junctions [29, 31]. The result is a slowing of conduction and an increase in repolarization dispersion in the atria, which constitute the structural and/or electrical substrate for the onset and/or maintenance of AF [30].

It should be noted that atrial remodeling refers to any persistent changes in atrial structure and/or function [13, 37]. Atrial structural remodeling includes inflammation, cell hypertrophy, atrial dilatation, apoptosis, and fibrosis, which together contribute to abnormal formation and conduction of electrical impulses as an arrhythmogenic substrate [3, 35]. It is also known that hemodynamic atrial overload in AF causes RAAS activation, which is associated with endothelial damage and recruitment of cytokine-secreting inflammatory cells [11].

Atrial fibrosis is thought to alter both the overall expression of gap junction proteins and their distribution along the cell membrane, causing a decrease in intercellular communication [4]. In addition, acquired risk factors for AF, especially cardiovascular disease, also influence atrial electrical and/or structural remodeling, which accounts for approximately 50% [13]. Finally, atrial remodeling can be caused by AF itself, leading to electrophysiological, contractile, and structural changes [10, 30].

In recent years, familial and population genetic studies of AF have led to the discovery of transcription factors as potentially important factors involved in atrial remodeling that contributes to arrhythmia susceptibility. Transcription factors can create a proarrhythmogenic substrate in pulmonary veins and atria. However, further studies are needed to fully characterize the links between these proteins and the pathogenesis of AF, which could potentially lead to the development of new treatments for arrhythmias.

GENETIC RISK ASSESSMENT OF AF

Genetic testing is useful to confirm the diagnosis as well as for differential diagnosis, recurrence risk calculation and prenatal diagnosis in families with known genetic variants of AF [5, 7]. The differential diagnosis should consider the presence of reversible causes of AF in the patient, especially metabolic disorders, and cardiovascular disease [13]. According to the recent Expert Consensus of the European Heart Rhythm Association / Heart Rhythm Society / Asia-Pacific Heart Rhythm Society / Latin American Heart Rhythm Society [38], the clinical value and applicability of genetic testing in AF is primarily considered from a prognostic standpoint and should be aimed at early identification of high-risk patients, which may contribute to the reduction of cardiovascular complications and mortality with adequate therapeutic options. The eligibility criteria for genetic testing for suspected familial form of AF are [2, 38]: 1) the presence of ECG-documented signs of AF; 2) a clinical picture of AF as the main clinical manifestation (phenotype) with early onset (before 60 years of age); 3) identification of a family history of at least one sick family member of the first or second degree of consanguinity. Genetic testing of *SCN5A, KCNQ1, MYL4,* and *TTN* truncating variants can be performed in all patients younger than 60 years of age with an established diagnosis of familial AF based on a review of the patient's medical history, family history, and ECG characterization [38].

GWAS studies have identified common variants in more than 100 genetic loci responsible for the development of AF. Several studies have attempted to incorporate genotype into de novo AF prediction models [18]. In this regard, the AF-PRS (atrial fibrillation polygenic risk score) was developed in 2013 to identify individuals at high risk of AF, its clinical outcomes, and to predict rhythm control therapy [17, 39].

This assessment consisted of 12 risk alleles at nine loci associated with isolated AF. Although the AF-PRS score is calculated based on multiple variants to identify a population at high risk of developing AF, a few prerequisites must be met [5]. First, the GWAS must be large enough to identify all common variants associated with AF. Second, there must be sufficient power to reproduce the AF-PRS in the validation dataset. AF-PRS score has been shown to predict the occurrence of AF than the association of clinical risk factors [8, 13] more clearly.

It has been shown that when the AF-PRS score was added to the basic model for predicting the development of AF in 20,000 women without cardiovascular disease, the area under the predictive value curve increased to 0.74 [13]. A PRS analysis of AF with 6.6 million variants in more than 500,000 patients found that 6.1% of the general population had a 3-fold higher risk of developing AF [40]. Identification of individuals with a 3-fold increased risk of developing AF is potentially «actionable» and may lead to increased screening and earlier therapeutic intervention and prevention of progression to persistent or permanent forms of AF [8].

It has been shown that multiple single nucleotide polymorphisms can improve the prediction of the devel-

opment of AF, including asymptomatic AF, and ischemic stroke [8]. AF-PRS assessment also has potential value as an indicator of anticoagulant therapy [40]. In addition, AF-PRS was as powerful as arterial hypertension in assessing clinical outcomes of AF [41]. No intergenic interaction regarding susceptibility to AF was detected.

The value of AF-PRS in predicting the recurrence of AF after treatment of AF was also evaluated. The presence of either of two single nucleotide polymorphisms, rs2200733 and rs10033464, on chromosome 4q25 has been shown to be an independent predictor of AF recurrence in patients undergoing electrical cardioversion [20]. Similarly, in patients with AF who underwent catheter ablation (CA), the presence of either of the same two single nucleotide polymorphisms increased the risk of early recurrence of AF (after ≤7 days) by 2-fold and late recurrence of AF (after 3-6 months) by 4-fold [24].

In addition, AF-PRS calculation based on analysis of 127 genetic variants, identified patients with a 2-fold increased likelihood of cardioembolic stroke [41]. In another study, calculation of AF-PRS with 32 variants in more than 50,000 patients with cardiovascular disease showed a 4-fold increase in stroke incidence in patients with high genetic risk, compared with a relatively «low risk» CHA_2DS_2-VASc score of 2.57 [41].

It should be noted that prediction models for the development of AF based on genetic information are not yet judged to be sufficiently convincing to distinguish between people at low and high risk of AF because of testing of a small number of variants, pleiotropy of AF genes, and the interaction of these genes with external risk factors.

TREATMENT OF FAMILIAL AF

Current therapies for rhythm control in AF include drug therapy and CA [35]. This paper reviews the genetic approach to therapy in familial AF and the prospects for gene therapy for AF.

General principles of gene therapy for AF

Among the potential cardiac arrhythmias that can be treated with gene therapy, AF is the most intensively studied [2, 42]. Advantages of gene therapy for AF include tissue specificity with fewer side effects, and possibly increased therapeutic efficacy [42]. For clinical practice, the safety of using gene therapy, the optimal way to deliver genetic material into the heart, and the establishment of long-term gene expression for myocardial modification are of great importance [43, 44]. Therefore, effective, and prolonged gene therapy for AF requires the development of innovative approaches to expand the therapeutic options.

Gene delivery can be accomplished using viral or non-viral vectors with varying degrees of gene inclusion and expression [20, 44]. Adenovirus and adeno-associated viruses are currently the most used viral vectors for cardiac gene therapy [45]. Viral vectors are live viruses, and their advantages include incorporation of genetic material into the genome of the target tissue as well as minimally invasive delivery through the bloodstream. A basic non-viral vector directly injected into the myocardium consists of a DNA plasmid containing the gene of interest, with or without other coating agents to improve DNA uptake by cells [44]. The advantages of plasmid DNA administration include a limited cellular and antibody-mediated immune response, allowing for repeated treatment.

There are several methods of gene delivery to the left atrium: epicardial injection of a plasmid carrying the gene of interest combined with electroporation; epicardial viral gene delivery and epicardial gene staining [6]. The gene staining technique is the optimal method for delivering target genes to the pulmonary veins and left atrium. The successful solution of endocardial gene staining technique may be the most appropriate way for electrophysiologists to perform gene therapy [44]. Using this technique, almost 100% of cells examined transmurally had evidence of gene transfer [42].

Although there is no «perfect» vector or delivery method that can target, integrate, and safely express genes in the myocardium in a «seamless» manner, the development of both viral and non-viral vectors and the creation of safer and more effective gene therapies for AF continues.

Genotypic approach to AF therapy

Variability in response to pharmacologic and non-pharmacologic therapy has been established in patients with AF [20, 32]. For example, some patients are free of AF for long periods of time with antiarrhythmic therapy, while others require repeated AF ablation within a few weeks [3]. The limited success of rhythm control therapy in AF is partly due to an incomplete understanding of the pathophysiologic mechanisms [10].

Recognizing that common genetic variants increase susceptibility to AF reinforces the possibility that they may also modulate response to rhythm control therapy. One of the first pharmacogenetic studies investigated whether there was a response to antiarrhythmic therapy (AAT) in symptomatic AF modified by the ACE I/D polymorphism [20]. This polymorphism, associated with increased ACE activity and cardiac fibrosis, was a significant predictor of AAT ineffectiveness in patients with early-onset AF. Patients with ACE genotype I/I showed a pronounced reduction of symptoms on the background of therapy, while in patients with genotype D/D the response to AAT was weak. In addition, we found that the single nucleotide polymorphism rs10033464 on chromosome 4q25 was an independent predictor of successful rhythm control in patients carrying the ancestral allele, having a fourfold increased chance of maintaining sinus rhythm. It has also been shown that the activity of flecainide is increased in patients with AF and β1AR Arg389Arg genotype, while heart rate control is achieved at lower doses of this drug [43].

F.Syeda et al. [19] showed that variable expression of PITX2 not only modulates atrial resting membrane potential, but also confirms the clinical observation that flecainide is more effective in suppressing AF than sotalol. In addition, patients carrying the variant allele rs10033464 responded better to treatment with class I versus class III antiarrhythmic drugs.

Overall, studies investigating the role of AF-PRS genetic risk for predicting the efficacy of AAT in AF are scarce. This is partly due to the growing importance of catheter ablation of AF and the lack of need to assess the response to AAT using AF-PRS [2, 20]. At the same time, with the expected increase in the need for rhythm control therapy for stroke prevention, there is great potential in the

application of AF genetic risk assessment for the management of AAT in the general population [10]. It is important to emphasize that almost all pharmacogenetic studies evaluating the response to AAT in AF have not been replicated and their effects are modest, reinforcing the need for randomized clinical trials before such approaches can be implemented in clinical practice.

Predicting the recurrence of AF after CA based on genetic testing may help identify patients who are indicated for regular clinical and electrocardiographic follow-up. For example, it has been shown that like the risk of first-onset AF, PITX2 was a major factor in the recurrence of AF after CA [32]. While clinical and echocardiographic variables could not predict recurrence, any variant alleles were associated with early and late recurrence of atrial arrhythmias after CA [46]. Another study confirmed the predictive value of ACE I/D polymorphism in the occurrence of early recurrence of AF after CA [32]. DD genotype and left atrial enlargement were found to be significantly associated with recurrence of AF. These studies have shown that genes involved in the pathogenesis of AF may not only predict risk of AF but also response to therapy.

The rs751141 variant in the *EPHX2* gene (encodes epoxyeicosatrienoic acids, which are involved in the modulation of cardiac ion channels) has also been shown to be associated with an increased risk of AF recurrence after CA [25]. Since nitric oxide has been implicated in modulation of cardiac vagus nerve activity and cardiac remodeling, the rs1799983 polymorphism in the *NOS3* gene has also been shown to be associated with early recurrence of AF after CA [24].

However, the value of screening for incident rare variants as predictors of recurrent AF after CA remains questionable. For example, rare variants in cardiac sodium channel genes, *SCN5A* and *SCN1B-4B*, were not significantly associated with CA outcome [25]. Despite some controversial points, AF-PRS assessment is a promising approach for predicting the efficacy of AF treatment in clinical practice.

Therapeutic targets of gene therapy for AF

Given the multifactorial origin of AF, different therapeutic targets for gene therapy of AF have been identified depending on their contribution to the re-entry mechanism [43]: shortened AP (ion channels, autonomic modulation) or slowed conduction (gap junctions, structural remodeling).

It should be noted that gene therapy aimed at modifying the electrical substrate of AF by reducing the expression of the fast potassium current I_{Kr} by inhibiting the *KCNH2* gene promotes atrial AP prolongation, increases their refractoriness and prevents AF [43]. It has also been shown that gene therapy leading to increased expression of L-type calcium channels either through up-regulation or by adding a highly expressed copy of the gene may be effective in preventing the occurrence of AF. In this case, T-type calcium channel blockers are the most effective compared to sodium, potassium and L-type calcium channel blockers.

Kv1.5 potassium channels are another potential target for gene therapy of AF [42-44], which regulate I_{Kur} current and lead to selective prolongation of atrial AP. It has been shown that Kv1.5 channel knockdown or knockout can have therapeutic effects without the need for repeated antiarrhythmic treatment. Some drugs that act at the atrial level, such as AVE 0118, have been shown to affect I_{Kur} current in the atrial auricles, shortening the duration of AP in chronic AF. In addition, inhibition of the potassium channel Task-1, which is an atrial-selective regulator of AP duration, is an attractive target for antiarrhythmic therapy in AF, especially in patients with heart failure [47]. Thus, therapeutic agents targeting ion channels may be useful in an early cardioversion strategy.

It has been demonstrated experimentally that restoring the structure/function of connexins may be useful in the treatment of AF [31, 44]. Connexin-40 and connexin-43 gene transfer using the epicardial staining method has been shown to significantly improve protein expression and localization, increase the concentration of gap junctions, and thereby cause improved conduction and reduced risk of AF [28, 29].

One effective treatment strategy for familial AF is to attenuate parasympathetic impulses (signaling). The left atrium, especially its posterior wall, is known to have a denser parasympathetic innervation compared to other atrial regions [48]. It has been demonstrated experimentally that stimulation of the cervical portion of the left vagus nerve causes shortening of the atrial refractory period and increased vulnerability to AF, whereas local pharmacologic blockade is protective [31]. It has also been found that gene therapy of AF by inhibiting the primary effector molecules of the Gαi/Gαo system attenuates the vagus nerve-induced shortening of the atrial refractory period and thus reduces the inducibility of AF [43].

Gene-based strategies to modify the structural substrate of AF involve suppression of inflammation and oxidative stress in the atria and consequently cellular fibrosis and apoptosis [43]. The main sign of age-related fibrosis is the activation of beta-transforming growth factor TGF-β [47]. A.Kunamalla et al. [49], in an experimental model of AF tried to modulate atrial fibrosis by delivery of dominant-negative TGF-β type II receptor to the posterior part of the left atrium. Therapy targeting TGF-β resulted in decreased fibrosis and reduced AF inducibility compared with the control group.

It has also been shown that transduction of lentivirus against miRNA206 into the superior left ganglionic plexus caused suppression of apoptosis, prolongation of AP and decreased AF inducibility [44]. Gene therapy for AF targeting cellular apoptosis involves suppression of caspase-3 activity, which can be inhibited by small or short interfering RNA (siRNA). In an experiment, treatment with an adenovirus vector containing siRNA resulted in suppression of apoptotic activity in the atrium and delayed the onset of persistent AF [42].

Active oxygen species (AOS) generated by oxidative stress have multiple interactions with several known triggers of AF, modulation of which has high therapeutic potential [37]. It has been shown that patients with AF have lower nitric oxide bioavailability than those without AP [43]. In addition, high levels of AOS are associated with enhanced TGF-β signaling, and the presence of atrial fibrosis [37]. AOS can damage mitochondrial DNA, caus-

ing myocyte calcium overload and electrical remodeling, leading to AF. Finally, high levels of AOS correlate with increased oxidation of calmodulin-dependent kinase II, which is associated with altered calcium cycling (turnover) and hence atrial electrical remodeling. Thus, oxidative stress-induced AOS are a compelling and multilevel target of AF therapy.

CONCLUSION

Given the relatively high prevalence of familial AF in the population, it is relevant to assess the potential risk of AF among relatives of a patient with isolated AF, and if genetic predisposition is suspected, it is advisable to perform genetic testing. Therefore, further studies are needed, primarily to test the clinical utility of information on family history of AF in addition to established risk factors for the development of AF. It also seems important to conduct genotype-phenotype association studies irrespective of allele frequency.

The response to antiarrhythmic therapy and CA of AF is known to be partially modulated by shared genetic variability; therefore, the development of a comprehensive clinical and genetic risk scale will allow the use of genetic data for the management of patients with FP. It should be noted that one of the most challenging aspects of AF treatment is the heterogeneity of genetic, structural, and electrical abnormalities that lead to the development of AF. Therefore, the use of targeted genetic alterations for personalized drug therapy of AF is a relevant problem. Currently, intensive experimental studies of suitable therapeutic targets for gene therapy of AF and the implementation of their results into clinical practice in patients with familial AF, as well as the development of effective and safe methods of gene therapy are ongoing. Given the economic impact of the AF epidemic, even small changes in therapeutic efficacy can result in substantial improvements for patients and the health care system.

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