The role of connexin40 in atrial fibrillation

Sevasti-Maria Chaldoupi, Peter Loh, Richard N.W. Hauer, Jacques M.T. de Bakker, and Harold V.M. van Rijen*

1Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, The Netherlands; 2Interuniversity Cardiology Institute of the Netherlands, Utrecht, The Netherlands; 3Heart Failure Center, Academic Medical Center University of Amsterdam, The Netherlands; and 4Department of Medical Physiology, Division Heart and Lungs, University Medical Center Utrecht, Alexander Numan Building, Yalelaan 50, 3584 CM Utrecht, The Netherlands

Received 21 January 2009; revised 3 June 2009; accepted 14 June 2009; online publish-ahead-of-print 17 June 2009

1. Introduction

Atrial fibrillation (AF) is the most commonly encountered sustained arrhythmia in man with a variable clinical presentation.1 Besides AF secondary to hypertension, coronary artery diseases, valvular abnormalities, cardiothoracic surgery, cardiomyopathy, inflammatory or infiltrative processes, endocrine disorders, and drug abuse, idiopathic AF is observed in 30% or more of patients.1 In addition, Cx40 gene mutations or polymorphisms give an inherited predisposition to AF.

In general, the vulnerability to arrhythmias of the heart is determined by the combined presence of an arrhythmogenic substrate and initiating triggers, both of which can be modulated by the autonomic nervous system or drugs.2 In patients prone to AF, the triggered activity originates mainly from the thoracic veins.3 These triggers combined with an atrial arrhythmogenic electrophysiologic substrate, caused by reduced effective refractory period (ERP),4–7 enhanced spatial dispersion of refractoriness8 or abnormal atrial impulse conduction9,10 lead to the initiation of AF.

Connexin40 (Cx40) is a major gap-junction protein in the atrial myocardium. In the heart, gap junctions are responsible for cell-to-cell conduction of the action potential. In several cardiac diseases, the expression of connexins is changed and is associated with increased propensity for arrhythmias. Atrial fibrillation (AF) is the most common arrhythmia in man with a diverse clinical presentation, different underlying mechanisms, and difficult treatment. The vulnerability to arrhythmias of the heart is determined by the combined presence of an arrhythmogenic substrate and initiating triggers. The arrhythmogenic substrate is formed by reduced effective refractory period, enhanced spatial dispersion of refractoriness, or abnormal atrial impulse conduction. Initiating triggers of AF most frequently originate from firing foci in the pulmonary veins and/or superior caval vein. Prolonged episodes of AF result in electrical and structural remodelling that favours the reoccurrence or perpetuation of AF. This electrical remodelling embodies changes in Cx40 expression and distribution, both in the atrial myocardium itself and in the thoracic veins. In addition, Cx40 gene mutations or polymorphisms give an inherited predisposition to AF. This review focuses on the role of Cx40 in AF, showing that abnormal Cx40 expression is correlated with both trigger formation from the thoracic veins as well as enhanced vulnerability of the atrial myocardium to AF.

AF is a self-perpetuating progressive disease in which ‘AF begets AF’.6 Prolonged episodes of AF result in electrical and structural remodelling that favours the reoccurrence or perpetuation of AF. Fast atrial rhythms and AF give rise to electrical remodelling, i.e. changes in ion-, and gap-junction channel expression.4–7 Structural remodelling, detected at a later stage, involves changes in mitochondrial size and the disruption of sarcoplasmic reticulum at the subcellular level, myocardial cell hypertrophy at the cellular level, and fibre disarray and increased collagen deposition at the tissue level.5

Electrical remodelling associated with AF lead to changes in the ERP.4–7 As part of this electrical remodelling, changes in gap junctions and connexins in AF have been reported,11–14 but this do not fall into a consistent pattern. Gap junctions are clusters of transmembrane channels that link adjoining cells and mediate cell-to-cell electrical coupling and communication. They are formed by the joining of two connexons (=hexameric hemi-channels), which are composed of six integral membrane subunits, connexins (Cx), that surround the central aqueous pore.15 In the human heart, four main isoforms are expressed.16 Cx43 is expressed in all chambers of the heart, but predominantly in the ventricles, Cx45 is found in the conduction system of the heart and at low levels in the

---

KEYWORDS
Connexins;
Atrial fibrillation
atrial and ventricular working myocardium,17 and Cx37 is located in the endothelial gap junctions in many vessels. Finally, Cx40 is expressed mainly in the atrial working myocardium, the conduction system, and the vasculature. Cx40 was first described in a range of animal species,18,19 and subsequently mapped to human chromosome 1. It became apparent that Cx40 was expressed in the atrioventricular conduction system20,21 and abundantly expressed in the atrial but not in the ventricular gap junctions.22,23 Recently, a new connexin was described in the mouse heart, i.e. Cx30.2 (the human equivalent is Cx31.9) which in mice seems responsible for slowing of impulse conduction in the atrioventricular node.24 However, the role of Cx31.9 in the human heart is unclear, for it is not detectable in the human cardiac conduction system.25

Several reviews described the mechanisms of AF26–29 or changes in Cx expression in cardiac disease.30,31 This review focuses on the role of Cx40 in AF, showing that the abnormal Cx40 expression is correlated to both trigger formation from the thoracic veins as well as enhanced vulnerability of the atrial myocardium to AF.

2. Contribution of Cx40 to the atrial electrical propagation

Cell-to-cell coupling by gap junctions is an essential determinant in uniform and successful propagation of the action potential and determined by the distribution and specific properties of connexins throughout the myocardium.32 In the human atria, Cx40 and Cx43 are the major connexins,16 and several experimental studies attempted to elucidate their role in myocardial conduction. Studies in Cx43 haploinsufficient mice have shown that P-wave duration is not affected by Cx43 levels, suggesting unchanged conduction velocity (CV) in the atria.33 Even levels of only 10% Cx43 did not significantly increase P-wave duration in the atria of mice.34 These studies indicated that Cx43 is not a principal determinant for atrial impulse conduction in the presence of normal Cx40 levels.

Several studies in Cx40 knockout mice (Cx40−/−) indicated that Cx40 is the dominant connexin for impulse conduction in the atria and the conduction system as summarized in Table 1. The majority of these studies demonstrated that full deficiency for Cx40 prolonged P-wave, PQ/PR interval, QRS, and QTc duration in the surface electrocardiogram.35–42 Epicardial mapping revealed that the prolonged P-wave and PQ/PR-interval were due to reduced CV in the atria, while the prolonged QRS complex was caused by right bundle branch block and reduced CV in the left bundle branch.42,43

Typically, Cx40 knockout mice were susceptible to atrial tachyarrhythmias.35–38,42 Although in mice, all studies are unambiguous, in respect to electrical propagation, Beauchamp et al.44 showed in synthetic strands of neonatal and foetal murine atrial cardiomyocytes that Cx40 deletion (Cx40−/−) was associated with increased electrical propagation velocity and genetic deletion of Cx43 (Cx31.9−/−) produced a decrease in propagation velocity. In addition, a study that investigated the correlation between Cx40 and Cx43 expression in the atria and the atrial conduction properties in humans showed that the propagation velocity in the atria is related to the interactions between Cx40 and Cx43 expression.45 A higher expression of immunodetectable Cx40 in the right atrium, in the presence of Cx43, reduced CV, while Cx43 alone was not directly correlated with propagation properties. The ratio of Cx43 to total Cx immunosignal (Cx43/[Cx40 + Cx43]) was directly and the ratio of Cx40 to total Cx ([Cx40/(Cx40 + Cx43)]) was inversely related to propagation velocity. These findings may be explained by the fact that heterotypic Cx40/Cx43 gap-junction channels may be present, which have much lower conductance than either Cx40 or Cx43 homotypic gap-junction channels.36,47 These data, however, are in contrast with the previously discussed mouse data, in which haploinsufficiency for Cx40 did not alter atrial impulse conduction and full deficiency for Cx40 is associated with lower impulse conduction. The apparent discrepancy between Cx40−/− mouse data and the latter in vitro and human data may be explained by specific expression patterns in adult mouse myocytes,44 and the fact that in transgenic animals altered expression of genes other than those targeted may occur.

3. Role of Cx40 distribution in maintenance of atrial fibrillation

The first studies to investigate the role of gap junctions in AF were carried out in animal models of AF (Table 2). Induction of persistent AF (lasting >2 months) in a goat model was

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Studies on Cx40 knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author (year)</td>
<td>SCL</td>
</tr>
<tr>
<td>Simon et al. (1998)39</td>
<td>↓↓</td>
</tr>
<tr>
<td>Kirchhoff et al. (1998)38</td>
<td>↓↓</td>
</tr>
<tr>
<td>Hagendorff et al. (1999)37</td>
<td>↑</td>
</tr>
<tr>
<td>Verheule et al. (1999)42</td>
<td>↓↓</td>
</tr>
<tr>
<td>Bevilacqua et al. (2000)36</td>
<td>↑</td>
</tr>
<tr>
<td>Tamaddon et al. (2000)40</td>
<td>↓</td>
</tr>
<tr>
<td>VanderBrink et al. (2000)31</td>
<td>↓</td>
</tr>
<tr>
<td>Van Rijen et al. (2001)43</td>
<td>↓</td>
</tr>
<tr>
<td>Bagwe et al. (2005)35</td>
<td>↓</td>
</tr>
<tr>
<td>Mean</td>
<td>↓</td>
</tr>
</tbody>
</table>

↓↓, unchanged; ↑, increased; ↓, decreased; ‘?’, difficult to attain conclusion/mean; SCL, sinus-node cycle length; PR, PR-interval; QRS, QRS duration; QTc, corrected QT-interval; Pdur, P-wave duration; AF, atrial fibrillation; AT, atrial tachycardia; NS, not studied; CV, conduction velocity; AH, atrial-His time; HV, His-ventricle time; RBBB, right bundle branch block; LBB, left bundle branch.
reported to lead to heterogeneous spatial distribution of Cx40, while the expression of Cx43 remained unchanged. 12 Heterogeneous expression was defined as the non-uniform labelling pattern of Cx40: patches of cells virtually devoid of Cx40, while the expression of Cx43 remained unchanged. 12 This heterogeneous distribution occurred after 2 weeks of high-rate atrial pacing. 48 Interestingly, discontinuation of AF resulted in reverse remodelling and gradual normalization of the altered distribution pattern of gap junctions and Cx40 expression. 49 Total Cx40 protein levels were unchanged or reduced in long-lasting AF with unchanged levels of Cx40 mRNA. 12,48,50

In humans, several studies investigated connexin expression and distribution during AF (Table 2) under different conditions. During sinus rhythm (SR), gap junctions are expressed mainly at the true end of the cells, 51–54 although side-to-side disposition can also be common in the atria. 54 During AF, however, Cx40 expression was predominantly side-to-side 51,53,54 and heterogeneously distributed, 11,53,55–57 while the expression of N-cadherin and Desmoplakin remained normal. 53 In patients with ischaemic heart disease and no history of arrhythmias, post-operative AF was more likely when pre-existing higher levels of Cx40 protein were present. 52 Other studies, however, that investigated changes in Cx40 expression and distribution in patients with long-lasting AF (at least 3 months and thus atrial remodelling) showed inconsistent results with respect to the amount of Cx40 protein level during AF. Some of those studies showed that the Cx40 protein levels were increased with lateralized expression in the atria, independent of AF aetiology. 54,58 Other studies found that the expression of Cx40 during AF was significantly reduced, 11,53,55,59 whereas various studies showed no difference in Cx40 protein expression levels between patients with SR and long-lasting AF 11,56,57 or that Cx40 expression levels were dependent on extracellular Ca 2+ level. 51

These seemingly different findings from the studies to date may in part be due to the different methodological approaches and experimental design. First, both polyclonal rabbit anti-rat Cx40 (S15) and anti-human Cx40 specific antibodies (Y2IY) were used in different studies for the quantification of Cx40. However, almost all studies pointed out that Cx40 gap junctions were heterogeneously distributed in the atria of patients with AF (Table 2). Such an expression of connexins may result in heterogeneous intercellular coupling leading to conduction defects and non-uniform anisotropic characteristics that can facilitate re-entrant circuits and therefore predispose to atrial tachycardias. 61 A second effect of inhomogeneous uncoupling might be the increased dispersion in refractoriness. Uncoupling was shown to lead to a dramatic increase in activation recovery intervals. 62

---

**Table 2.** Cx40 expression and distribution in atrial myocardium during atrial fibrillation

<table>
<thead>
<tr>
<th>AF type</th>
<th>Cx40 protein level</th>
<th>mRNA</th>
<th>Cx40 distribution</th>
<th>Remarks</th>
<th>Author (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goats, AF &gt; 2 months</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous Cx43 ↓, ventricular fibrillation</td>
<td>↓</td>
<td>Van der Velden et al. (1998) 12</td>
</tr>
<tr>
<td>Goats, AF 1, 2, 4, 8, 16 week</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous Cx43 ↓, Cx40/Cx43 ratio ↓</td>
<td>↓</td>
<td>Van der Velden et al. (2000) 56</td>
</tr>
<tr>
<td>Goats, AF 13.9 ± 5.2 week</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Thijssen et al. (2002) 50</td>
</tr>
<tr>
<td>Post-AF (CAD)</td>
<td>↑</td>
<td>↑</td>
<td>Heterogeneous lateralization</td>
<td>↑</td>
<td>Dupont et al. (2001) 52</td>
</tr>
<tr>
<td>CAF &gt; 1 year (CAD, MVD, AVD)</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous lateralization Cx43 ↓, LA 63 ± 11.9 mm</td>
<td>↓</td>
<td>Polontchouk et al. (2001) 54</td>
</tr>
<tr>
<td>CAF &gt; 1 year (mini-maze)</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous lateralization Cx43 ↓, LA 55 ± 7 mm</td>
<td>↓</td>
<td>Kostin et al. (2002) 53</td>
</tr>
<tr>
<td>CAF &gt; 5 months (MVD)</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous lateralization Cx43 ↓, LA 55 ± 7 mm</td>
<td>↓</td>
<td>Nao et al. (2003) 55</td>
</tr>
<tr>
<td>CAF &gt; 6 months (MVD)</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous lateralization Cx43 ↓, LA 55 ± 7 mm</td>
<td>↓</td>
<td>Kanagaratnam et al. (2004) 51</td>
</tr>
<tr>
<td>LAF and AF (MVD)</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous lateralization Cx43 ↓, LA 55 ± 7 mm</td>
<td>↓</td>
<td>Wetzel et al. (2005) 58</td>
</tr>
<tr>
<td>Persistent AF &gt; 3 months</td>
<td>↓</td>
<td>↓</td>
<td>Homogenous LA 45 ± 4 mm</td>
<td>↓</td>
<td>Wilhelm et al. (2006) 59</td>
</tr>
<tr>
<td>CAF &gt; 3 months (MVD)</td>
<td>↓</td>
<td>↓</td>
<td>Heterogenous LA 54 ± 9.8 mm</td>
<td>↓</td>
<td>Takeuchi et al. (2006) 57</td>
</tr>
<tr>
<td>Post-AF (CAD)</td>
<td>↓</td>
<td>↓</td>
<td>Heterogeneous Cx43 ↓, fibrosis ↑, LA 38.3</td>
<td>↓</td>
<td>Li et al. (2008) 56</td>
</tr>
<tr>
<td>CAF &gt; 1 year (CAD, MVD, AVD, mini-maze)</td>
<td>↑</td>
<td>↑</td>
<td>Heterogeneous lateralization Cx43 ↑, (unaffected of Ca 2+), LA 49 ± 1</td>
<td>↑</td>
<td>Dhein et al. (2008) 51</td>
</tr>
</tbody>
</table>

→, unchanged; ↑, increased; ↓, decreased; NS, not studied; CAD, coronary artery disease; LA, left atrium; CAF, chronic atrial fibrillation; LAF, lone atrial fibrillation; MVD, mitral valve defect; AVD, aorta valve defect; NF-kB, nuclear factor κB.
Interestingly, in heart failure, the heterogeneous expression of Cx43 is associated with both dispersion of impulse conduction\textsuperscript{63,64} and dispersion of refractoriness.\textsuperscript{63}

4. The role of Cx40 in the arrhythmogenic properties of the thoracic veins in atrial fibrillation

Triggers emerging from the thoracic veins, i.e. the pulmonary veins (PVs)\textsuperscript{3} and the superior vena cava (SVC),\textsuperscript{65} are important factors in the initiation and perpetuation of AF.\textsuperscript{3,65} So called myocardial sleeves, the extensions of atrial myocardium into the PVs and SVC, are well described\textsuperscript{66} and identified as the underlying substrate for these triggers. The mechanisms behind the ectopic activity from the thoracic veins are thought to be based on either automaticity or micro-re-entry and the possible role of Cx40 in this arrhythmogenic behaviour has been subject of several studies (Table 3).

Ectopic activity from the PVs is the most prominent triggers for AF.\textsuperscript{3} Saito \textit{et al.}\textsuperscript{67} studied the anatomy of the PVs in human hearts and showed that the myocardial cells in the PVs are separated from the muscular media of the veins suggesting that the trigger from the PVs must originate from the myocardial sleeves. Arora \textit{et al.}\textsuperscript{68} showed that the conduction at the proximal part of canine PVs was considerably slower than in the remaining left atrium (LA). Also in canine PVs, Hocini \textit{et al.}\textsuperscript{69} found zones of activation delay that were related to sudden changes in fibre direction that could result in micro-re-entry and suggested that Cx distribution and expression might play a supplementary role. Evidence for this was provided by Verheule \textit{et al.}\textsuperscript{13} who demonstrated that, although the myocytes in the canine PVs were similar to those in LA, the gap junctions in the myocardial sleeves expressed mainly Cx43 and that the levels of Cx40 were significantly lower than in the LA. On the other hand, spontaneous electrical activity was observed in PVs isolated from guinea pig hearts.\textsuperscript{70} The response of this spontaneous activity to perivascular nerve stimulation was similar to that seen at sinoatrial node (SAN).\textsuperscript{70} Node-like cells were identified in myocardial sleeves of PVs of adult rats and the intercalated disk of those cells were composed of small gap-junctional specializations comparable to those seen in the SAN.\textsuperscript{71,72} Furthermore, these myocardial sleeves correspond to areas of the conduction system in embryonic myocardium and originate from the sinus venosus segment of the heart from which also the SAN originates.\textsuperscript{73,74} Cells of the PV sleeves originate from mesenchymal stem cells and are not recruited from atrial cells.\textsuperscript{75}

Studies in dogs with atrial remodelling due to mitral valve regurgitation or rapid atrial pacing demonstrated that Cx40 protein expression in the PVs was downregulated which may be important for the maintenance of AF.\textsuperscript{76,77} In summary, in PVs, both automaticity and activation delay resulting in micro-re-entry may form the source of triggers for AF. Gap-junction remodelling seems to play an important role in two ways. First, the fact that the PVs contain autorhythmic cells, which share a sinus nodal-like gap-junction expression, may be able to drive the atria. Secondly, abnormal and discontinuous gap-junction expression with rapid changes in fibre direction may facilitate micro-re-entry, resulting in pre-excitatory triggering of the atrial myocardium.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Type of the study</th>
<th>Cx40 protein level</th>
<th>Distribution</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeh et al (2001)\textsuperscript{80}</td>
<td>Mongrel dogs (n = 8)</td>
<td>Centre area Cx43, periphery Cx40</td>
<td>Homogenous</td>
<td>$\uparrow$ (2 week), $\uparrow$ (6–8 week), ERP, CV, AF inducibility</td>
</tr>
<tr>
<td>Lee et al (2005)\textsuperscript{83}</td>
<td>RAP canine dogs</td>
<td>NS</td>
<td>NS</td>
<td>ERP, NS, Cx expression similar to SAN</td>
</tr>
<tr>
<td>Verheule et al (2002)\textsuperscript{13}</td>
<td>Mongrel dogs (n = 8)</td>
<td>Homogenous</td>
<td>NS</td>
<td>ERP, NS, Cx expression similar to SAN</td>
</tr>
<tr>
<td>Sun et al (2008)\textsuperscript{76}</td>
<td>MR dogs with AF (n = 5)</td>
<td>NS</td>
<td>NS</td>
<td>ERP, NS, Cx expression similar to SAN</td>
</tr>
<tr>
<td>Zhang et al (2008)\textsuperscript{77}</td>
<td>RAP beagle dogs with AR (n = 11)</td>
<td>NS</td>
<td>NS</td>
<td>ERP, NS, Cx expression similar to SAN</td>
</tr>
</tbody>
</table>

S. Chaldoupi \textit{et al.}
In the SVC, myocardial sleeves also extend from the RA-SVC junction up to 2–5 cm into the SVC.78,79 Yeh et al.80 studied the electrical properties of the SVC in canine hearts that are structurally comparable to human SVC. In SVC myocardial sleeves, gap junctions composed of Cx40, Cx43, and Cx45 were exclusively found at the intercalated disk. The distribution of Cx40 was homogeneous throughout the myocardial sleeves. In the proximal part of the sleeves, atypical areas were present that extended to 1 cm distally from the RA-SVC junction where Cx43 expressed in the centre, surrounded by Cx40 spots. Interestingly, the Cx expression pattern of these atypical areas is analogous to that reported for SAN of dogs.81 Automativeness of SVC may determine the RA-SVC junction up to 2–5 cm into the SVC.78,79 Yeh et al.80 studied the electrical properties of the myocardial sleeves in the SVC. The SAN-like Cx expression pattern may favour the exit of activity from spontaneously active cells to surrounding myocardium. The investigators not only demonstrated pacemaker activity of some of the cardiomyocytes in the SVC, but also showed the presence of delayed after depolarization in a large percentage of cells suggesting that the triggered activity plays an additional role in the ectopic activity in SVC.

In a dog model of rapid atrial pacing, pacing for 2 and 6–8 weeks resulted in electrical and structural remodelling of the myocardial sleeves in SVC.14 Beside changes in size, arrangement, and proliferation of myocytes, there was perceptible remodelling of the gap-junction distribution and Cx expression. During the first 2 weeks of continuous pacing, an upregulation of Cx43 and a downregulation of Cx40 occurred and Cx were redistributed to the lateral borders of individual cardiomyocytes. Rapid pacing also resulted in shortening of the refractory period, decreased CV of the myocardial sleeves, and increased vulnerability to AF.83 Alterations in cell-to-cell coupling may contribute to this observed change in velocity.84

The specific pattern of Cx expression, combined with the intrinsic automativeness of SVC myocytes may determine the mechanism for triggers emerging from the SVC under normal conditions. During AF, however, the alteration in the expression and distribution of Cx40 may change the electrical characteristics of the SVC and cause inhomogeneous and discontinuous propagation of the impulse as well as activation delay through the myocardial sleeves, a substrate supporting re-entry.

5. The Cx40 gene mutations and polymorphisms and atrial fibrillation predisposition

AF has a variable clinical presentation and character, which may result from a genetic substrate with different gene mutations and/or polymorphisms. Familial forms of AF, due to gene mutations or polymorphisms, have been described. An autosomal dominant trait of AF was first described in a small family in Spain by Brugada et al.85 Loss- or gain-of-function mutations in several potassium (K+) channel genes (KCNQ1, KCNE2, KCNJ2, and KCNA5) have been described in familial forms of AF.86–89 Furthermore, relatives of probands with lone AF are at substantially increased risk of developing this arrhythmia as well, suggesting a hereditary origin.90–94 Besides gene mutations, many gene polymorphisms are described in idiopathic AF, such as polymorphisms of angiotensin converting enzyme,95 potassium channels gene polymorphisms,96 and sodium channels gene polymorphisms.97

Besides ion channels, abnormalities in the Cx40 gene (GJA5) have been reported to be associated with atrial arrhythmias. Groenewegen et al.98 were the first to connect the Cx40 gene with a rare atrial arrhythmia in humans. They showed that atrial standstill, a disease characterized by lack of electrical and mechanical activity of the atria, was due to a combination of a rare polymorphism of the promoter of the Cx40 gene at nucleotides, –44 (G → A) and +71 (A → G), that occurs in 7% in the population, with a novel mutation in the sodium channel gene SCN5A. They demonstrated that patients with either the SCN5A mutation or the presence of that Cx40 polymorphism did not show evidence of atrial standstill. Only the combined effect of those genetic variants led to an additive and progressive pathological response and the presence of atrial standstill.

Firouzi et al.99 were the first to correlate the vulnerability for AF to this Cx40 promoter polymorphism in patients without structural heart disease in the absence of atrial remodelling. They compared the electrophysiological characteristics of 30 patients with supraventricular tachycardia and very rare episodes of AF with those without evidence of AF or history of episodes with irregular heartbeat. They illustrated that homozygous carriers of the minor haplotype (–44AA/+71GG) were more prone to both inducibility of AF by programmed electrical stimulation and spontaneous occurrence of AF episodes. This predisposition to initiation of AF appeared to be related to enhanced dispersion of atrial refractoriness. Juang et al.100 showed the same relation in Taiwanese patients with paroxysmal or permanent AF. They showed that patients with AF (n = 173) had a significantly higher Cx40 (–44AA/+71GG) genotype frequency compared with the control group (232 patients). Finally, Gollob et al.101 studied 15 patients with idiopathic AF with early onset (+45 years) and refractory to pharmacological therapy. They identified four novel, three somatic, and one germline, heterozygous mutations in the Cx40 gene in four of those patients.

Functional studies in cell lines have shown that the promoter activity of Cx40 with the minor allele (–44A/+71G) was significantly reduced compared with the major allele (–44G/+71A).96,102 To what extend the levels or distribution of Cx40 protein is decreased due to this polymorphism is unclear yet. Further studies are needed to correlate the presence of the minor allele (–44A/+71G) to changes in the Cx40 protein level. Atrial sample of patients with mutations in the Cx40 gene showed abnormal gap-junction formation with intracellular accumulation of Cx40.103 This abnormal expression and distribution of Cx40 protein presumably leads to heterogeneous impulse propagation that may increase AF vulnerability.

6. Factors modulating Cx40

Since abnormal expression of Cx40 is closely related to the vulnerability to AF, normalization of Cx40 expression may be a successful therapeutic avenue. However, little is known about the mechanisms that underlie atrial remodelling in general (e.g. reviewed by Brundel et al.102) and
factors that specifically modulate Cx40 function during AF. Recently, Sarrazin et al. showed that oral administration of n-3 polyunsaturated fatty acids can result in reduced vulnerability to induction of AF in dogs. This protection of n-3 polyunsaturated fatty acids can result in reduced vulnerability to induction of AF. It was shown that ZP123 prevents the reduction of atrial CV, which Cx modifier is most successful for the treatment of AF. They were mainly studied to explore their antiarrhythmic character in the ventricular myocardium which resulted in reduced dispersion of action potential duration and enhancement of gap-junctional conductance. The effect of rotigaptide (also known as ZP123, a potent AAP analog with improved plasma stability) on AF was subject of several studies. In a rabbit model of volume overload-induced AF, rotigaptide increased atrial CV, however, without the reduction of AF vulnerability. The expression levels of Cx43 and Cx40 were down regulated in the model, but remained unaltered after rotigaptide treatment. Similarly, in a dog model of AF resulting from either atrial or ventricular tachy-pacing, rotigaptide improved CV, without altering AF duration or vulnerability. However, in a dog model of AF due to myocardial ischemia, the addition of rotigaptide prevented ischemia-induced conduction slowing and reduced AF duration. Similar results were obtained in a canine sterile peri-carditis model, in which GAP-134 (like rotigaptide an AAP analog) was able to reduce AF. Additional studies in rats showed that ZP123 prevents the reduction of atrial CV during metabolic stress, however, the drug had no effect under normal physiological conditions. Many studies have shown that Cx function can be modified. Further research is needed to establish which Cx modifier is most successful for the treatment of AF.

7. Conclusion

In this review, we have focused on the role of Cx40 in the initiation and maintenance of AF. Direct manipulation of the Cx40 amount in mice is related to conduction slowing and vulnerability to AF (in the absence of other structural or electrical remodelling). In patients with AF, Cx40 expression is heterogeneous, which may lead to abnormal impulse formation and conduction, which may form the substrate for AF. Altered Cx40 expression and distribution in the myocardial sleeves of the thoracic veins may be the substrate for abnormal impulse formation and/or micro-re-entry, underlying the trigger for AF initiation. At the same time, a genetic predisposition, due to Cx40 gene polymorphism or other short mutations, seems also to be related to the initiation of AF. One last point of discussion may be the redundancy of connexins in the heart. For Cx43, it has been shown that a 50% reduction in Cx43 does not alter ventricular impulse conduction and mice haploinsufficient for Cx40 do not have an electrical phenotype. Therefore, changes in Cx40 expression alone may not be sufficient for conduction slowing and arrhythmogenesis. The aetiology of AF is not equal between patients, as a result of which the contribution of Cx40 abnormalities may vary. Other factor, such as enhanced fibrosis, as often found during AF, may be prerequisite for conduction slowing and enhanced arrhythmogenesis. Finally, therapies involving enhancement of Cx function using antiarrhythmicogenic peptides have been proved successful, underlining the role of Cx40 as potential target for AF therapy.

Conflict of interest: none declared.

References


