A novel GATA5 loss-of-function mutation underlies lone atrial fibrillation

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Abstract. Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is associated with significantly increased morbidity and mortality. Cumulative evidence highlights the importance of genetic defects in the pathogenesis of AF. However, AF is of remarkable heterogeneity and the genetic determinants of AF in a vast majority of patients remain illusive. In this study, the coding exons and splice junctions of the GATA5 gene, which encodes a zinc-finger transcription factor essential for normal cardiogenesis, were sequenced in 118 unrelated patients with lone AF. The available relatives of the index patient carrying an identified mutation and 200 unrelated ethnically-matched healthy individuals used as controls were genotyped. The functional effect of the mutant GATA5 was characterized in contrast to its wild-type counterpart using a luciferase reporter assay system. As a result, a novel heterozygous GATA5 mutation, p.W200G, was identified in a family with AF inherited as an autosomal dominant trait. The mutation was absent in 200 control individuals and the altered amino acid was completely conserved evolutionarily across species. Functional analysis showed that the mutation of GATA5 was associated with a significantly decreased transcriptional activity. These findings provide novel insight into the molecular mechanism involved in AF, suggesting potential implications for the early prophylaxis and gene-specific therapy of AF.

Introduction

Atrial fibrillation (AF) is the most common form of cardiac arrhythmia encountered in clinical practice and the main cause of arrhythmia-related hospitalizations, accounting for approximately 1/3 of hospitalizations for heart rhythm disorders (1). The prevalence of AF is estimated to be 1% in the general population, and it increases strikingly as the population ages, with a prevalence of approximately 0.1% in individuals younger than 55 years of age, roughly 4% among those over 60 years and nearly 10% in those aged 80 years and older (2). According to the Framingham Heart Study, the lifetime risk of developing AF is at least 25% for subjects who have reached the age of 40 (3). AF is associated with substantially increased cardiovascular morbidity and mortality; it increases the risk of stroke by 3 to 5-fold, imposing a large economic burden on national healthcare systems around the world and a deleterious impact on the quality of life of patients (4). The risk of cerebrovascular thromboembolism ascribed to AF also increases abruptly with advancing age, rising from 1.5% at age 50-59 years up to 23.5% at age 80-89 years (4). AF also independently increases the risk of congestive heart failure and the risk of mortality by 1.5 to 2-fold compared with cases in sinus rhythm (5). Additionally, AF is responsible for complications such as adverse hemodynamics, reduced exercise capacity, impaired cognitive function or dementia and tachycardia-induced cardiomyopathy (6). AF has traditionally been regarded as an acquired disease secondary to miscellaneous cardiac or systemic conditions, including hypertension, coronary artery disease, congenital heart disease, rheumatic heart disease, chronic pulmonary heart disease, cardiomyopathy, cardiology, obstructive sleep apnea, diabetes mellitus, hyperthyroidism and electrolyte imbalance (1). However, in 30-45% of AF patients, no established risk factors are identified by routine procedures, and such AF is defined as ‘idiopathic’ or ‘lone’ (1), of which at least 15% have a positive family history, so termed familial AF (7). Growing evidence has documented the familial aggregation of AF and an enhanced susceptibility to AF in the close relatives of patients with AF, indicating that hereditary defects may play an important role in the pathogenesis of AF in a subset of patients (8-14). Genome-wide
linkage analysis with polymorphic genetic markers mapped multiple susceptibility loci for AF on human chromosomes 10q22, 6q14-16, 11p15.5, 5p13, 10p11-q21 and 5p15, of which AF-causing mutations in 2 genes, KCNQ1 on chromosome 11p15.5 and NUP155 on chromosome 5p13, were identified and functionally characterized (15-21). Additionally, a genetic scan of candidate genes revealed a long list of AF associated genes, including KCNE2, KCNE3, KCNE5, KCNH2, KCNJ2, KCNA5, SCN5A, SCN1B, SCN2B, SCN3B, NPPA, GJA1 and GJA5 (22-37). Nevertheless, AF is a genetically heterogeneous disease and the genetic determinants for AF in a large proportion of patients remain unclear.

Emerging evidence underscores the crucial role for several transcription factors, including NKX2-5, GATA4 and GATA6, in the proper cardiogenesis (38-40) and mutations in these genes have been causally linked to congenital cardiovascular anomalies and AF (41-56). GATA5 is another member of the GATA family and its expression and function overlap with those of GATA4 and GATA6 during cardiac development, particularly in the regulation of target gene expression synergistically with NKX2-5 (57,58), suggesting the potential association of functionally compromised GATA5 with AF.

To assess the prevalence of GATA5 mutations in patients with lone AF and to explore the mechanism by which mutated GATA5 causes or confers susceptibility to AF, the coding exons and exon/intron boundaries of GATA5 were sequenced in patients with lone AF in contrast to control individuals and the functional effect of the mutant GATA5 was characterized in comparison with its wild-type counterpart using a luciferase reporter assay system.

Materials and methods

Study population. A cohort of 118 unrelated patients with lone AF was identified among the Han Chinese population in China. The available relatives of the index patients were enrolled and a total of 200 ethnically-matched unrelated healthy individuals were recruited as controls. Peripheral venous blood specimens were prepared and clinical data including medical records, electrocardiogram and echocardiography reports were collected. The study subjects were clinically classified using a consistently applied set of definitions (7,53). Briefly, diagnosis of AF was made by a standard 12-lead electrocardiogram demonstrating no P waves and irregular R-R intervals regardless of clinical symptoms. Lone AF was defined as AF occurring in individuals <60 years of age without other cardiac or systemic diseases by physical examination, electrocardiogram, transthoracic echocardiogram and extensive laboratory tests. Familial AF was defined as the presence of documented lone AF in 2 or more first- or second-degree relatives. Relatives with AF occurring at any age in the setting of structural heart disease (hypertensive, ischemic, myocardial or valvular) were classified as ‘undetermined’ for having an inherited form of AF. The ‘undetermined’ classification was also used if documentation of AF on an electrocardiogram tracing was lacking in relatives with symptoms consistent with AF (palpitations, dyspnea and light-headedness), or if a screening electrocardiogram and echocardiogram were not performed, irrespective of the symptoms. Relatives were classified as ‘unaffected’ if they were asymptomatic and had a normal electrocardiogram. Paroxysmal AF was defined as AF lasting >30 sec that terminated spontaneously. Persistent AF was defined as AF lasting >7 days and requiring either pharmacological therapy or electrical cardioversion for termination. AF that was refractory to cardioversion or that was allowed to continue was classified as permanent. The study protocol was reviewed and approved by the local institutional ethics committee and written informed consent was obtained from all research participants prior to conducting investigation.

Genotyping. Genomic DNA from all participants was extracted from blood lymphocytes with the Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA). Initially, the whole coding sequence and splice junctions of the GATA5 gene were screened in 118 unrelated patients with lone AF. Subsequently, genotyping GATA5 in the available relatives of the index patient carrying an identified mutation and in the 200 ethnically-matched unrelated healthy individuals used as controls was performed. The referential genomic DNA sequence of GATA5 was derived from GenBank (accession no. HM015595). With the assistance of online Primer3 software (http://frodo.wi.mit.edu), the primer pairs used to amplify the coding exons (exons 2-7) and intron-exon boundaries of GATA5 by polymerase chain reaction (PCR) were designed as shown in Table I. PCR was carried out using HotStar Taq DNA Polymerase (Qiagen, Hilden, Germany) on a PE 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with standard conditions and concentrations of reagents. Amplified products were purified with the QiAquick Gel Extraction kit (Qiagen). Both strands of each PCR product were sequenced with a BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) under an ABI PRISM 3130XL DNA analyzer (Applied Biosystems). The sequencing primers were those designed previously for specific region amplifications. DNA sequences were viewed and analyzed with the DNA Sequencing Analysis Software v5.1 (Applied Biosystems). The variant was validated by resequencing of an independent PCR-generated amplicon from the subject and met our quality control threshold with a call rate exceeding 99%.

Alignment of multiple GATA5 protein sequences across species. The multiple GATA5 protein sequences across various species were aligned using an online program MUSCLE, version 3.6 (http://www.ncbi.nlm.nih.gov/).

Construction of recombinant pcDNA3.1-hGATA5 expression plasmid. Human fetal cardiac tissue specimens were previously collected and preserved in RNAlater RNA Stabilization Reagent (Qiagen). Total-RNA was prepared using an RNeasy Protect Mini kit (Qiagen). Reverse transcription was performed with Oligo(dT)20 primer using SuperScript III reverse transcriptase (Invitrogen LifeTechnologies, Carlsbad, CA, USA). The full-length wild-type cDNA of the human GATA5 gene, including partial 5‘- and 3‘-untranslated regions, was PCR amplified using pfUUltra high-fidelity DNA polymerase (Stratagene, La Jolla, CA, USA). The primer pairs used for the specific amplification of the GATA5 transcript were: forward, 5‘-GTA GCT AGC CAC CGC CGT GCC
CTG CCG-3' and reverse, 5'-GAT GCG GCC GCT GTT CCC CTG ACA TGG GC-3'. The PCR fragment with a length of 1,296 base pairs was doubly digested by endonuclease NheI and NotI. The digested product was fractionated by 1.5% agarose gel electrophoresis, purified with the Qiagen Gel Extraction kit (Qiagen) and then subcloned into pcDNA3.1 (Promega Corporation) to form a eukaryotic expression vector, pcDNA3.1-hGATA5.

Site-directed mutagenesis. The identified mutation was introduced into the wild-type GATA5 using a QuikChange II XL Site-Directed Mutagenesis kit (Stratagene) with a complementary pair of primers. The mutant was sequenced to confirm the desired mutation and to exclude any other sequence variations. The 4 pairs of primers used to confirm the mutant clone were: primer 1, forward, 5'-TAA TAC GAC TCA CTA TAG GG-3' and reverse, 5'-TGG TAG GCA CTG CCG TCT, CG-3' (product size, 443 bp); primer 2, forward, 5'-CCT TCC CCC CGG CTT CTC AGC AGC-3' and reverse, 5'-CGA GGA CAG GCC CTT AGG GC-3' (product size, 431 bp); primer 3, forward, 5'-GCA ATG CCT GCC GCC TCT AC-3' and reverse, 5'-GAG CTG TCA GTC CTG GCG AC-3' (product size, 340 bp); primer 4, forward, 5'-CCA GAC ACG GAA GCC GAA GC-3' and reverse, 5'-CTG CCG TTC TGA TGG CTG CCT GA-3' (product size, 426 bp).

Reporter gene assays. The atrial natriuretic factor (ANF)-luciferase reporter gene, which contains the 2600-bp 5'-flanking region of the ANF gene, namely ANF(-2600)-Luc, was kindly provided by Dr Ichiro Shiojima, from the Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan. HEK-293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. The ANF(-2600)-Luc reporter construct and 0.04 µg of plasmid pGL4.75 (hRluc/CMV; Promega Corporation) to form a eukaryotic expression vector, pcDNA3.1-hGATA5.

Statistical analysis. Data are expressed as the means ± SD. Continuous variables were tested for normality of distribution and Student's unpaired t-test was used for comparison of numeric variables between 2 groups. Comparison of the categorical variables between 2 groups was performed using Pearson's χ² test or Fisher's exact test when appropriate. A two-tailed P-value <0.05 was considered to indicate statistically significant differences.

Results

Characteristics of the study subjects. A total of 118 unrelated patients with lone AF and a cohort of 200 ethnically-matched unrelated healthy individuals used as controls were enrolled and clinically evaluated. None of them had overt traditional risk factors for AF. There were no significant differences between patient and control groups in baseline characteristics including age, gender, body mass index, blood pressure, fasting blood glucose, serum lipid, left atrial dimension, left ventricular ejection fraction, heart rate at rest, as well as life style (data not shown). At the time of the present study, 8 patients were also diagnosed with hypertension in accordance with the criterion that the average systolic or diastolic blood pressure (2 readings made after 5 min of rest in the sitting position) was ≥140 or ≥90 mm Hg, respectively, but at the time of initial diagnosis of AF, their blood pressures were normal. The baseline clinical characteristics of the 118 patients with lone AF are summarized in Table II.

GATA5 mutation. Direct sequencing of the coding exons and exon-intron boundaries of the GATA5 gene was conducted after PCR amplification of genomic DNA from each of the 118 patients with lone AF. A heterozygous GATA5 mutation was identified in 1 out of 118 unrelated patients, with a prevalence of ~0.85% for GATA5 mutation. In particular, a substitution of guanine (G) for thymine (T) in the first nucleotide of codon 200 (c.598T>G), predicting the transition of tryptophane (W) into glycine (G) at amino acid 200 (p.W200G) was identified in a patient with positive family history. The sequence chromatograms showing the detected heterozygous GATA5 mutation of c.598T>G compared with the corresponding control sequence are shown in Fig. 1. A schematic diagram of GATA5 depicting the putative structural

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer (5’→3’)</th>
<th>Reverse primer (5’→3’)</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>GGC ATA AGC TCG GGC GCT GG</td>
<td>TGG GCC CCG AGA CTG TGG AG</td>
<td>648</td>
</tr>
<tr>
<td>3</td>
<td>TGA CGA AAG CCC CCA GGC TC</td>
<td>CCC CAG GGG CTC TGG TGT CA</td>
<td>375</td>
</tr>
<tr>
<td>4</td>
<td>CCG CAA GGC CGA CCT GAG TC</td>
<td>CCG CTC CTC CCC AGC CTC TT</td>
<td>312</td>
</tr>
<tr>
<td>5</td>
<td>GGG AAT CCA GCT CCA CGG GC</td>
<td>CTG GAG GCA CCG AAG GCC AC</td>
<td>331</td>
</tr>
<tr>
<td>6</td>
<td>GCC TGC GTG GTG ACC GTG AG</td>
<td>GGT GTG TCC AGC CCA CCT GC</td>
<td>370</td>
</tr>
<tr>
<td>7</td>
<td>CCC CCA TGC CAT TCC AGG GC</td>
<td>GGG GCC TGC TGG TCT CTG CT</td>
<td>402</td>
</tr>
</tbody>
</table>
Table II. Baseline clinical characteristics of the 118 patients with lone atrial fibrillation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. or quantity</th>
<th>Percentage or range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>65</td>
<td>55</td>
</tr>
<tr>
<td>Age at first diagnosis of atrial fibrillation (years)</td>
<td>52.84</td>
<td>32-59</td>
</tr>
<tr>
<td>Type of atrial fibrillation at presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>82</td>
<td>69</td>
</tr>
<tr>
<td>Persistent</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Permanent</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Positive family history of atrial fibrillation</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>History of cardioversion</td>
<td>38</td>
<td>32</td>
</tr>
<tr>
<td>History of cardiac pacemaker</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>78.31</td>
<td>52-176</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125.73</td>
<td>95-138</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85.02</td>
<td>70-88</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.38</td>
<td>20-24</td>
</tr>
<tr>
<td>Left atrial diameter (mm)</td>
<td>38.17</td>
<td>28-42</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>64</td>
<td>50-78</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.40</td>
<td>4-6</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.52</td>
<td>3-5</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.33</td>
<td>1-2</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amiodarone</td>
<td>89</td>
<td>75</td>
</tr>
<tr>
<td>Aspirin</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Warfarin</td>
<td>72</td>
<td>61</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Digoxin</td>
<td>48</td>
<td>41</td>
</tr>
</tbody>
</table>

Figure 1. Sequence electropherograms of GATA5 in the proband and in a control individual. The arrow indicates the heterozygous nucleotides of T/G in the proband (mutant) or the homozygous nucleotides of T/T in a control individual (wild-type). The square denotes the nucleotides comprising a codon of GATA5.

domains and location of the mutation identified in AF patients is presented in Fig. 2. The missense mutation was not found in the control population nor was it reported in the NCBI's SNP database (http://www.ncbi.nlm.nih.gov/SNP). Genetic screening of the available family members demonstrated that the mutation was present in all affected living family members, but absent in unaffected family members examined. Analysis of the pedigree showed that the mutation cosegregated with AF transmitted as an autosomal dominant trait in the family with complete penetrance. The pedigree structure of the family is illustrated in Fig. 3. The phenotypic characteristics and results of genetic screening of the affected family members are listed.
in Table III. Congenital atrial septal defect was confirmed by medical records of previous catheter-based repairs in 2 AF patients (I-2 and II-8).

**Alignment of multiple GATA5 protein sequences.** A cross-species alignment of GATA5 protein sequences showed that the altered amino acid was completely conserved evolutionarily, as presented in Fig. 4, suggesting that the amino acid is functionally important.

**Transcriptional activity of the GATA5 mutant.** The transcriptional activation characterization of the mutated GATA5 in HEK-293 cells was examined using one of its direct cardiac downstream target genes, ANP, as a luciferase reporter and the activity of the ANP promoter was presented as fold activation of Firefly luciferase relative to Renilla luciferase. The same amounts of wild-type (0.4 µg) and W200G-mutant GATA5 (0.4 µg) activated the ANP promoter by ~13- and 4-fold, respectively. When the same amount of wild-type GATA5 (0.2 µg) was cotransfected with W200G-mutant GATA5 (0.2 µg), the induced activation of the ANP promoter was ~6-fold. These results suggest that the GATA5 mutation has a significantly reduced transcriptional activation compared with its wild-type counterpart (Fig. 5).

**Discussion**

In the present study, a novel heterozygous GATA5 mutation of p.W200G identified in a family with lone AF is reported. This missense mutation of GATA5 was present in all the affected family members examined but was absent in the unaffected family members available and in the 400 normal chromosomes from a matched control population. A cross-species

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Table III. Phenotypic characteristics and status of the GATA5 mutation of the affected pedigree members.

<table>
<thead>
<tr>
<th>Identity</th>
<th>Gender</th>
<th>Age at time of study (years)</th>
<th>Phenotype</th>
<th>AF (classification)</th>
<th>Age at diagnosis of AF (years)</th>
<th>Electrocardiogram</th>
<th>Echocardiogram</th>
<th>Subject Information</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-2</td>
<td>F</td>
<td>58</td>
<td>Permanent</td>
<td>58</td>
<td>30</td>
<td>118</td>
<td>98</td>
<td>440</td>
<td>NA</td>
</tr>
<tr>
<td>II-3</td>
<td>M</td>
<td>52</td>
<td>Persistent</td>
<td>52</td>
<td>43</td>
<td>104</td>
<td>93</td>
<td>465</td>
<td>+/-</td>
</tr>
<tr>
<td>II-4</td>
<td>F</td>
<td>32</td>
<td>Paroxysmal</td>
<td>32</td>
<td>40</td>
<td>90</td>
<td>78</td>
<td>420</td>
<td>+/-</td>
</tr>
<tr>
<td>III-4</td>
<td>F</td>
<td>32</td>
<td>Permanent</td>
<td>32</td>
<td>40</td>
<td>90</td>
<td>78</td>
<td>420</td>
<td>+/-</td>
</tr>
</tbody>
</table>

*Age at death. AF, atrial fibrillation; F, female; M, male; QTc, corrected QT interval; LAD, left atrial dimension; LVEF, left ventricular ejection fraction. + indicates present and - denotes absent.
hybridization, stability and sequence specificity of protein-DNA binding as consensus motif, while the N-terminal ZF is responsible for required for DNA sequence recognition and binding to the transcriptional activity of GATA5. The C-terminal ZF is predicted to consist of 2 transcriptional activation domains (TADs), 2 adjacent zinc fingers (ZFs) and 1 nuclear localization signal (NLS). The 2 TADs are both essential for the (TADs), 2 adjacent zinc fingers (ZFs) and 1 nuclear localization signal (NLS). The 2 TADs are both essential for the

Figure 4. Alignment of multiple GATA5 protein sequences across species. The altered amino acid of p.W200 is completely conserved evolutionarily across species.

Figure 5. Functional defect resulted from GATA5 mutation. Activation of ANP-luciferase reporter in HEK-293 cells by GATA5 wild-type (WT) or mutant (W200G), alone or in combination, showed significantly reduced transcriptional activation by mutant protein. Experiments were performed in triplicate and mean and standard deviations are shown. *P<0.001 and **P<0.005, when compared with wild-type GATA5.

alignment of multiple GATA5 protein sequences showed that the altered amino acid was completely conserved evolutionarily. Functional analysis demonstrated that the p.W200G mutation of GATA5 was associated with a significantly decreased transcriptional activity. Therefore, it is highly likely that functionally impaired GATA5 is involved in the pathogenesis of AF in this family. To the best of our knowledge, this is the first report on the relationship between GATA5 loss-of-function mutation and susceptibility to AF. These results expand the spectrum of mutations in GATA5 linked to AF and provide significant insight into the molecular basis underlying AF.

GATA transcription factors are a group of DNA binding proteins characteristic of preferential binding to the consensus DNA sequence GATA of target gene promoters. The GATA family comprises 6 members (GATA1 to GATA6), of which GATA4, GATA5 and GATA6 are expressed in various mesoderm and endoderm-derived tissues, particularly in the embryonic and adult heart (39). GATA5 maps to human chromosome 20q13.33 by fluorescence in situ hybridization, which encodes a predicted 397-amino-acid protein (58). Compared with the functional domains of GATA4, GATA5 is predicted to consist of 2 transcriptional activation domains (TADs), 2 adjacent zinc fingers (ZFs) and 1 nuclear localization signal (NLS). The 2 TADs are both essential for the transcriptional activity of GATA5. The C-terminal ZF is required for DNA sequence recognition and binding to the consensus motif, while the N-terminal ZF is responsible for stability and sequence specificity of protein-DNA binding as well as transcriptional activation by GATA factors. Most of the protein-protein interactions of GATA factors are mediated by its C-terminal ZF. The NLS sequence is associated with the sub-cellular trafficking and distribution of GATA5. The GATA5 mutation of p.W200G identified in this study is located in the N-terminal ZF, thus it may be expected to exert influence on the transcriptional activity of GATA5.

It has been corroborated that GATA5 is an upstream regulator of multiple genes transcribed during embryogenesis and cardiac morphogenesis including the genes that encode atrial natriuretic peptide (ANP), brain natriuretic peptide, α-myosin heavy chain, β-myosin heavy chain and cardiac troponin C and I (39). Hence, the functional effects of the GATA5 mutation may be ascertained by analysis of the transcriptional activity of the ANP promoter in cells transfected with the GATA5 mutant in contrast to its wild-type counterpart. In this study, the functional role of the novel p.G200W mutation of GATA5 identified in our familial AF patients was characterized by transcriptional activity assays and the results demonstrated a significantly decreased transcriptional activity on a downstream gene. These findings indicate that haploinsufficiency resulting from GATA5 mutations is potentially an alternative pathophysiological mechanism involved in AF, although the functional roles of the recently reported AF related GATA5 mutations remain to be explored (59).

The findings that functionally impaired GATA5 predisposes to AF can be partially attributed to the abnormally developed pulmonary vein myocardium. The pulmonary venous vessel is ensheathed by a layer of myocardium termed pulmonary myocardial sleeve, which has been substantiated to be responsible for the initiation and perpetuation of AF by several potential arrhythmogenic mechanisms including intrinsic pacemaker activity and properties that facilitate re-entrance (60-62). Genetic-labeling lineage tracing studies have revealed that NKX2-5 is expressed in the atria and pulmonary myocardium and is crucial for the localized formation of the sinoatrial node during embryogenesis. NKX2-5 may function as a suppressor of the sinoatrial node lineage gene program, which limits pacemaker activity to the sinoatrial and atrioventricular nodes. When the NKX2-5 protein decreased in a hypomorphic model, the pulmonary cardiomyocytes switched to connexin40-negative, HCN4-positive cells, a nodal-like phenotype with pacemaker activity (61). In NKX2-5-null mouse embryos, HCN4 was activated in the entire embryonic heart tube, whereas connexin40 expression was inhibited, and ectopic pacemaker cells were observed throughout the heart tube (63). In humans, AF was observed as an isolated phenotype or as a part of compound phenotypes...
in patients carrying NKX2-5 mutations (45,64,65). Therefore, as a transcriptionally cooperative partner of NKX2-5, GATA5, when a dominant negative mutation occurs, may contribute to the formation of the pulmonary myocardium sleeve and the shift of the pulmonary myocardium to a sinoatrial node-like phenotype by reducing NKX2-5, hence creating an atrial electrophysiological substrate liable to AF.

There are some downstream genes transactivated by GATA5, and mutations in several target genes have been implicated in AF, including the genes that encode β-myosin heavy chain, atrial natriuretic peptide and gap junction protein connexin40 (32,33,35-37,66). Therefore, it is highly likely that mutated GATA5 confers susceptibility to AF by decreasing expression of target genes.

Markedly, congenital atrial septal defect has been documented in 2 AF patients harboring the p.G200W mutation of GATA5. Similar to our findings, congenital cardiovascular malformations were previously confirmed in AF patients carrying NKX2-5, GATA4 or GATA6 mutations (45,51-53,56,59). Considering some congenital cardiac structural defects may close spontaneously, we cannot rule out the possibility that some mutation carriers had minor cardiac septal defects that closed shortly after birth on their own. These observations indicate that AF may share a common genetic origin with congenital heart disease.

In conclusion, our findings provide novel insight into the molecular mechanism associated with AF, suggesting potential implications for early prophylaxis and gene-specific therapy of this common tachycardia.

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