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ABSTRACT

Background: Atrial fibrillation (AF) and atrial flutter (AFL) are common cardiac conduction disorders affecting many people. Recent studies on sporadic cases of AF/AFL showed a significant association of the single nucleotide polymorphism rs2200733T with the disease, suggesting a genetic factor in the development of the disease.

Objectives: To determine the association of rs2200733 with AF/AFL derived from an Italian population sample.

Subjects: 78 patients with AF/AFL and 348 controls took part in the study.

Design: Genetic case–control study.

Results: The results indicate that there is a positive, significant association between the rs2200733 T allele and patients with AF/AFL of Italian origin (allelic p < 0.001 with OR = 2.17).

Conclusion: These results derived from a sample of the Italian population agree with previously reported findings from an Icelandic study, which also found that the minor allele rs2200733 was associated with AF/AFL disease.

Cardiac conduction disorders may depend upon a variety of predisposing factors, including developmental and congenital defects, acquired injuries or ischaemia of the conduction system and rarely inherited genetic defects that alter the electrophysiological properties of the heart.

Atrial fibrillation (AF) is the most common sustained arrhythmia (supraventricular tachyarhythmia).1 It is associated with increased cardiovascular morbidity and mortality and it is a leading cause of stroke.2 Other predisposing disease states include diabetes mellitus, essential hypertension (EH), congestive heart failure, valvular disease and myocardial infarction. The electrophysiological mechanism underlying AF may include the following events: myocardial electrical conduction and structural remodelling, genetic changes, ectopic electrical activity within the pulmonary veins and oxidative stress of the cardiac tissue.3 Thus, more than one mechanism may be involved in the onset of AF.

AF and atrial flutter (AFL) are closely related.4 AFL is of variable duration, and in many instances precedes the onset of AF.4 During AFL the heart upper chambers quiver, resulting in a beating five times faster than normal (usually 240–350 bpm).

Alcohol intake is significantly associated with patients with AF under age 60.5 In the 38-year follow-up Framingham Heart Study, EH and diabetes mellitus were found to be the only significant independent cardiovascular risk factors for AF after adjusting for age and predisposing conditions.6,7

Gudbjartsson et al in their study performed a genome-wide association study on a sample of the Icelandic population with 550 patients with AF and AFL and 4476 controls,8 and found three single nucleotide polymorphisms (SNPs) associated with the phenotype in the 4q25 locus. Interestingly, in this region are located the paired like homeodomain 2 (Pitx2) gene and the ankryin B (Ank B) gene. The Pitx2 gene, especially, was originally identified through screening of patients with Axenfeld–Rieger syndrome, an autosomal dominant disease causing craniofacial abnormalities.9–11 Cardiac abnormalities are uncommon in this syndrome; however, low levels of Pitx2 are required for correct cardiac development. Moreover, molecular studies have demonstrated that three different Pitx2 isoforms (Pitx2a, Pitx2b and Pitx2c) are expressed throughout development and are generated by alternative splicing and promoter usage.11–12 Recently, a fourth isoform (Pitx2d) has been found, although it only appears to be present in humans.12 Among all the isoforms, only Pitx2c is expressed asymmetrically within the left lateral plate mesoderm and the developing heart.13

Additionally, a long QT syndrome 4 (LQT4) was mapped to chromosome 4q25–27 in a large French family with autosomal dominant LQTS associated with sinus node dysfunction and atrial fibrillation.14 The study by Mohler and colleagues established the Ank B gene (4q25–27) as the first non-ion channel involved in LQTS and arrhythmias.15 Ankryin B is a protein of 220 kDa and it is required for normal expression of Na/K ATPase, Na/Ca exchanger and inositol trisphosphate receptor in cardiomyocytes.

Thus, these genes are interesting candidates for studying the genetics of AF/AFL and its mechanism of pathogenesis.

Among the SNPs investigated by Gudbjartsson et al in the Icelandic population study, rs2200733 showed the most robust association (p = 0.0000000016; odds ratio (OR) = 1.75). Furthermore, this association has been validated in other population studies with different genetic backgrounds from Sweden and the United States.8 No difference between men and women was seen; however, there was an increased OR when the Icelandic patients with AFL (n = 116) were considered separately from the patients with AF (OR = 2.60). In the Icelandic population, no association was detected with obesity, myocardial infarction and EH, suggesting that these covariates are not related to risks factors of AF.8

Familial clustering of AFL, AF and Wolff–Parkinson–White syndrome has been reported.16–18
The first locus for familial AF was identified on chromosome 10q22–24 in three different Spanish families.\(^20\) Mutations in the sodium ion channel (SCN5A) gene have been suggested to be a cause of idiopathic AF,\(^21\) and possibly of AFL, conduction disease, Brugada syndrome and sudden cardiac death.\(^22\)

Moreover, other evidence suggests that AF in parents increases the future risk for AF in their offspring.\(^23\) In this study, we genotyped SNP rs2200733 in sporadic cases of AF and AFL (n = 78) and in ethnically matched controls (n = 348) to determine whether this SNP is associated with AF/AFL diseases in an Italian population and whether we could consider it as a candidate genetic marker for investigated pathologies.

### Subjects and Methods

#### Subject characteristics

A total of 33 patients with AFL and 45 with AF were enrolled for the genetic analysis. The criterion for inclusion was consecutive patients with AF or AFL referred to our Institution (IRCCS Multimedica, Milan, Italy). Patients were symptomatic and had not been taking antiarrhythmic drugs for a week before admission. We performed genetic analysis in 78 arrhythmic subjects and 348 controls. For our analysis we carried out an electrophysiological study and catheter ablation of selected patients with AF and AFL referred to our Institution (IRCCS Multimedica, Milan, Italy). Patients were symptomatic and were free from antiarrhythmic drugs for a week before admission. We performed genetic analysis in 78 arrhythmic subjects and 348 controls. For our analysis we carried out an electrophysiological study and catheter ablation of selected patients with AF and AFL referred to our Institution (IRCCS Multimedica, Milan, Italy). Patients were symptomatic and were free from antiarrhythmic drugs for a week before admission. We performed genetic analysis in 78 arrhythmic subjects and 348 controls. For our analysis we applied a statistical approach following a \(\chi^2\) distribution to test inferences of association between the rs2200733 marker and the AF/AFL phenotype at both allelic and genotypic levels. The analysis was conducted for patients with AF and AFL as two separate groups and for all patients in one group. Analysis of rs2200733 showed a strong allelic and genotypic association with AF/AFL disease (table 1).

The minor allele frequency in affected subjects was 26.9% compared with 14.5% in unaffected controls (allelic \(p < 0.001\) and \(OR = 2.17\); genotypic \(p < 0.001\)). Interestingly, the value of \(OR\) increased when AFL was analysed separately (\(OR = 2.38\)), in agreement with the previous results published in an Icelandic population study.

#### DNA isolation and genotyping

DNA was extracted from blood samples of patients with AF/AFL and controls using a Qiamp DNA blood Midikit (Qiagen, Germantown, USA) according to the manufacturer's protocol.

Genotyping was performed using Taqman Assay. PCRs and post-PCR fluorescent measurements were carried out on an ABI7900 (Applied Biosystems, Foster City, USA). For genotyping, cluster plots were made of the fluorescent labels using SDS software (v2.2, Applied Biosystems). Spots falling outside a cluster were labelled as undetermined.

#### Statistical analysis

We used gPLINK\(^*\) to estimate allele and genotype frequencies and performed Hardy–Weinberg (HW) equilibrium tests. Furthermore, we performed the G test with Williams correction (a statistic following a \(\chi^2\) distribution) using software R (http://www.R-project.org (accessed 15 July 2008)) in order to compare allele and genotype frequencies between cases and controls. To assess significance, we considered a \(p\) value < 0.05. The OR relative to the allele frequencies and its 95% CI were also calculated.

### Results

#### Genetic association study

In our analysis, we carried out an electrophysiological study and catheter ablation of selected patients with AF and AFL referred to our Institution (IRCCS Multimedica, Milan, Italy). Patients were symptomatic and were free from antiarrhythmic drugs for a week before admission. We performed genetic analysis in 78 arrhythmic subjects and 348 controls. For our analysis we applied a statistical approach following a \(\chi^2\) distribution to test inferences of association between the rs2200733 marker and the AF/AFL phenotype at both allelic and genotypic levels. The analysis was conducted for patients with AF and AFL as two separate groups and for all patients in one group. Analysis of rs2200733 showed a strong allelic and genotypic association with AF/AFL disease (table 1).

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#### Discussion

For a long time, it has been known that AF and AFL have a close clinical relationship. Recent electrophysiological studies, particularly mapping studies, have significantly advanced our understanding of this relationship. Patients who primarily manifest AFL commonly also experience AF and vice versa.\(^4\) Additionally, recent studies have provided evidence of a genetic contribution to AF,\(^25\)\(^26\) Hence, we genotyped SNP rs2200733 (4q25) in sporadic cases of AF/AFL from Italy. In accordance with Gudbjartsson's study,\(^5\) the association with the T minor allele is significantly replicated in Italian sporadic cases of AF/AFL (allelic \(p < 0.001\) and \(OR = 2.17\); genotypic \(p < 0.001\)). Interestingly, the value of \(OR\) increases when AFL was analysed separately (\(OR = 2.38\)), in agreement with the previous results published in an Icelandic population study.

Our results showed that rs2200733 is significantly associated with sporadic investigated arrhythmias in Italian subjects.

Our future aim is to assess through the fine mapping approach the association of the studied region (4q25) in a larger number of subjects with AFL/AF compared with controls.

Moreover, rs2200733 is located in the 4q25 region. The Pitx2 gene, in particular, is found in this locus and the protein encoded by this gene is an interesting candidate for AFL/AF disease. In fact, Pitx2 is a bicoid-related homoeodomain transcription factor that plays a critical role in directing cardiac asymmetric morphogenesis. However, several aspects remain elusive. Also, the investigated SNP is located near to the Ank B gene. Ank B protein is expressed in both atrial and ventricular cardiomyocytes and loss of function mutation in this gene is thus likely to affect multiple aspects of the heart function (atrial and ventricular rhythm, function of the sinus and atrioventricular node). Thus, the second goal of future research is to identify

### Table 1

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>rs2200733 T Frequency</th>
<th>p Value</th>
<th>OR L95*</th>
<th>U95†</th>
<th>TT Frequency</th>
<th>CT Frequency</th>
<th>CC Frequency</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial flutter (n = 33)</td>
<td>0.29</td>
<td>0.003</td>
<td>2.38</td>
<td>1.34</td>
<td>4.22</td>
<td>0.15</td>
<td>0.27</td>
<td>0.58</td>
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<tr>
<td>Atrial fibrillation (n = 45)</td>
<td>0.26</td>
<td>0.006</td>
<td>2.02</td>
<td>1.2</td>
<td>3.98</td>
<td>0.11</td>
<td>0.29</td>
<td>0.60</td>
</tr>
<tr>
<td>AF and AFL (n = 78)</td>
<td>0.27</td>
<td>&lt;0.001</td>
<td>2.17</td>
<td>1.44</td>
<td>3.23</td>
<td>0.13</td>
<td>0.28</td>
<td>0.59</td>
</tr>
<tr>
<td>Controls (n = 348)</td>
<td>0.15</td>
<td>0.01</td>
<td>0.27</td>
<td>0.72</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Lower interval; †upper interval.
genetically the association of genes localised in the locus 4q25 as Pitx2 and Anke B and the mechanism for the pathogenesis of AFL/AF disease.

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Ethics approval: Ethics approval was obtained.

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