Association of rs2200733 at 4q25 with atrial flutter/fibrillation diseases in an Italian population

C Viviani Anselmi, V Novelli, R Roncarati, A Malovini, R Bellazzi, R Bronzini, G Marchese, G Condorelli, A S Montenero and A A Puca

Heart 2008;94;1394-1396
doi:10.1136/hrt.2008.148544

Association of rs2200733 at 4q25 with atrial flutter/fibrillation diseases in an Italian population

C Viviani Anselmi,[1] V Novelli,[1] R Roncarati,[1] A Malovini,[1,2] R Bellazzi,[2] R Bronzini,[1] G Marchese,[1] G Condorelli,[1] A S Montenero,[1] A A Puca[1]

[1] Istituto Ricovero Cura Carattere Scientifico "Multimedica", Milan, Italy; [2] University of Pavia, Pavia, Italy

Correspondence to:
Dr A A Puca, Unit of Genetics and Cardiovascular Research Institute, IRCCS Policlinico MultiMedica, Via Milanese 300, 20099 Sesto S Giovanni, Italy; puca@longevita.org

CVA and VN contributed equally to the work

Accepted 12 June 2008

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 tachyarrhythmia).[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.[1 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 homoeodomain 2* (*Pitx2*) gene and the *ankyrin 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 10] 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] 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] Ankyrin B is a protein of 220 kDa and it 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*,[8] 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.[16–19]

Table 1 Association of rs2200733 in with sporadic cases of atrial flutter (AFL) and atrial fibrillation (AF)

Phenotype	rs2200733 T Frequency	p Value	OR	L95*	U95†	TT Frequency	CT Frequency	CC Frequency	p Value
Atrial flutter (n = 33)	0.29	0.003	2.38	1.34	4.22	0.15	0.27	0.58	0.002
Atrial fibrillation (n = 45)	0.26	0.006	2.02	1.2	3.98	0.11	0.29	0.60	0.003
AF and AFL (n = 78)	0.27	<0.001	2.17	1.44	3.23	0.13	0.28	0.59	<0.001
Controls (n = 348)	0.15					0.01	0.27	0.72	

*Lower interval; †upper interval.

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 and common AFL who were referred to our institution for electrophysiological study and catheter ablation. All patients were symptomatic and had not been taking antiarrhythmic drugs for a week before their admission. Their mean (SD) age was 65.1 (12.0) for patients with AF and 62.4 (12.7) for those with AFL. A total of 348 healthy control subjects (average age 35) were enrolled for the study. The control group had no known medical condition.

The internal review board of Multimedica granted approval for this study. Also, all subjects involved signed an informed consent form. This study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

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[24] 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 χ^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 χ^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.

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.[23 25 26] Hence, we genotyped SNP rs2200733 (4q25) in sporadic cases of AF/AFL from Italy. In accordance with Gudbjartsson's study,[8] 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 we analyse patients with AFL separately (OR = 2.38) (table 1), in accordance with the Icelandic 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

genetically the association of genes localised in the locus 4q25 as *Pitx2* and *Ank B* and the mechanism for the pathogenesis of AFL/AF disease.

Acknowledgements: We thank Dr Natalia Rivera for her valuable help with writing the manuscript.

Funding: Italian Ministry of Health (1% funds), Italian Ministry of Research and University (FIRB). VN and RR are supported by a fellowship of the Doctorate School of Molecular Medicine, University of Milan, Italy.

Competing interests: None for this manuscript. However, for purposes of complete transparency, AAP has a non-funding affiliation with Elixir Pharmaceuticals.

Ethics approval: Ethics approval was obtained.

REFERENCES

1. **Medi C,** Hankey GJ, Freedman SB. Atrial fibrillation. *Med J Aust* 2007;**186**:197–202.
2. **Go AS,** Hylek EM, Phillips KA, *et al*. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. *JAMA* 2001;**285**:2370–5.
3. **Tsang TS,** Miyasaka Y, Barnes ME, *et al*. Epidemiological profile of atrial fibrillation: a contemporary perspective. *Prog Cardiovasc Dis* 2005;**48**:1–8.
4. **Waldo AL,** Feld GK. Inter-relationships of atrial fibrillation and atrial flutter mechanisms and clinical implications. *J Am Coll Cardiol* 2008;**51**:779–86.
5. **Marcus GM,** Smith LM, Whiteman D, *et al*. Alcohol intake is significantly associated with atrial flutter in patients under 60 years of age and a shorter right atrial effective refractory period. *Pacing Clin Electrophysiol* 2008;**31**:266–72.
6. **Aksnes TA,** Schmieder RE, Kjeldsen SE, *et al*. Impact of new-onset diabetes mellitus on development of atrial fibrillation and heart failure in high-risk hypertension (from the VALUE Trial). *Am J Cardiol* 2008;**101**:634–8.
7. **Lloyd-Jones DM,** Wang TJ, Leip EP, *et al*. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. *Circulation* 2004;**110**:1042–6.
8. **Gudbjartsson DF,** Arnar DO, Helgadottir A, *et al*. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature* 2007;**448**:353–7.
9. **Semina EV,** Reiter R, Leysens NJ, *et al*. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. *Nat Genet* 1996;**14**:392–9.
10. **Hjalt TA,** Semina EV. Current molecular understanding of Axenfeld-Rieger syndrome. *Expert Rev Mol Med* 2005;**7**:1–17.
11. **Schweickert A,** Campione M, Steinbeisser H, *et al*. Pitx2 isoforms: involvement of Pitx2c but not Pitx2a or Pitx2b in vertebrate left-right asymmetry. *Mech Dev* 2000;**90**:41–51.
12. **Cox CJ,** Espinoza HM, McWilliams B, *et al*. Differential regulation of gene expression by PITX2 isoforms. *J Biol Chem* 2002;**277**:25001–10.
13. **Campione M,** Ros MA, Icardo JM, *et al*. Pitx2 expression defines a left cardiac lineage of cells: evidence for atrial and ventricular molecular isomerism in the iv/iv mice. *Dev Biol* 2001;**231**:252–64.
14. **Schott JJ,** Charpentier F, Peltier S, *et al*. Mapping of a gene for long QT syndrome to chromosome 4q25-27. *Am J Hum Genet* 1995;**57**:1114–22.
15. **Mohler PJ,** Schott JJ, Gramolini AO, *et al*. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003;**421**:634–9.
16. **Light PE.** Familial Wolff-Parkinson-White Syndrome: a disease of glycogen storage or ion channel dysfunction? *J Cardiovasc Electrophysiol* 2006;**17**(Suppl 1):S158–61.
17. **Brugada R,** Tapscott T, Czernuszewicz GZ, *et al*. Identification of a genetic locus for familial atrial fibrillation. *N Engl J Med* 1997;**336**:905–11.
18. **Sarkozy A,** Brugada P. Sudden cardiac death and inherited arrhythmia syndromes. *J Cardiovasc Electrophysiol* 2005;**16**(Suppl 1):S8–20.
19. **Gillor A,** Korsch E. [Familial manifestation of idiopathic atrial flutter]. *Monatsschr Kinderheilkd* 1992;**140**:47–50.
20. **Roberts R.** Mechanisms of disease: genetic mechanisms of atrial fibrillation. *Nat Clin Pract Cardiovasc Med* 2006;**3**:276–82.
21. **Olson TM,** Alekseev AE, Liu XK, *et al*. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. *Hum Mol Genet* 2006;**15**:2185–91.
22. **Rossenbacker T,** Carroll SJ, Liu H, *et al*. Novel pore mutation in SCN5A manifests as a spectrum of phenotypes ranging from atrial flutter, conduction disease, and Brugada syndrome to sudden cardiac death. *Heart Rhythm* 2004;**1**:610–5.
23. **Fox CS,** Parise H, D'Agostino RB Sr, *et al*. Parental atrial fibrillation as a risk factor for atrial fibrillation in offspring. *JAMA* 2004;**291**:2851–5.
24. **Purcell S,** Neale B, Todd-Brown K, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;**81**:559–75.
25. **Arnar DO,** Thorvaldsson S, Manolio TA, *et al*. Familial aggregation of atrial fibrillation in Iceland. *Eur Heart J* 2006;**27**:708–12.
26. **Ellinor PT,** Yoerger DM, Ruskin JN, *et al*. Familial aggregation in lone atrial fibrillation. *Hum Genet* 2005;**118**:179–84.