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A sequence variant in *ZFHX3* on 16q22 associates with atrial fibrillation and ischemic stroke

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Abstract

We performed a genome-wide scan for sequence variants associating with atrial fibrillation in Iceland and followed up the most significant associations in samples from Iceland, Norway and USA. A sequence variant, rs7193343-T, in the *ZFHX3* gene on chromosome 16q22 associated significantly with atrial fibrillation (combined OR=1.21, P=1.4·10^{-10}). This variant also associates with ischemic

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stroke (OR=1.11, *P*=0.00054) and cardioembolic stroke (OR=1.22, *P*=0.00021) in a combined analysis of five stroke sample sets.

Atrial fibrillation (AF) is a common condition with a lifetime risk of one in four for men and women 40 years of age and older[1]. The disease carries significant mortality as well as morbidity and is a major risk factor for cardioembolic stroke (CES), one form of ischemic stroke (IS). AF increases the risk of stroke four to fivefold across all age groups and accounts for 10-15% of all IS[2].

We have previously reported on a genome-wide association study in Iceland identifying sequence variants close to the *PITX2* gene on chromosome 4q25 that confer risk of AF and atrial flutter (AFl)[3]. To search for additional variants that associate with AF, we have increased the sample size of this association study to 2,385 AF/AFl cases and 33,752 controls (see Supplementary Methods online for description of study groups). Genotyping was performed using the Illumina HumanHap300 and HumanHapCNV370 bead chips. After quality filtering, 303,136 SNPs were tested individually for their association with AF/AFl (quantile-quantile plot in Supplementary Figure 1).

Of the top ten SNPs from our genome-wide analysis, the seven most significant variants correspond to the previously reported signal on chromosome 4q25[3] (Supplementary Table 1). The remaining three SNPs have not been associated with AF/AFl before. In an attempt to follow up our findings we genotyped the three SNPs in three additional sample sets of European ancestry, from Iceland (989 cases and 2,027 controls), Norway (725 cases and 725 controls) and the US (735 cases and 729 controls). Two of the three SNPs did not associate significantly with AF/AFl in the follow-up samples and failed to reach genome-wide significance (Supplementary Table 2). The T allele of the third SNP, rs7193343, located on chromosome 16q22 (Table 1), showed genome-wide significant association with AF/AFl in the combined Icelandic sample set (OR=1.21, $P=9.2\cdot10^{-9}$). This association was subsequently replicated in the non-Icelandic samples (OR=1.21, *P*=0.0057). The combined effect of rs7193343-T in the discovery and three follow-up sets was OR=1.21 (95% CI: 1.14-1.28) with a corresponding *P* value of $1.4\cdot10^{-10}$.

We assessed the association of rs7193343-T with AF in a Han Chinese population from Hong Kong, consisting of 286 AF cases and 2763 controls. The association was not significant in this cohort although the direction of association was consistent with that in the European samples (OR=1.05, *P*=0.68). Notably, the T allele of rs7193343 is much more frequent in the Han Chinese population (the allelic frequency in controls is 0.68) than the samples of European descent (the allelic frequency in controls is between 0.14 and 0.20).

In our previous genome-wide study on AF/AFl, a stronger association was observed with the releatively small subset of individuals with a definite history of AFl than other cases[3]. We therefore tested rs7193343 in the subset of 160 Icelandic patients with a definite history of AFl. The association with AFl is similar to that with AF although it does not reach nominal significance on its own (OR=1.25, 95% CI: 0.96, 1.62, *P*=0.093).

We found no correlation between rs7193343 and obesity, hypertension or coronary artery disease in the Icelandic sample set (data not shown). This suggests that the association between rs7193343 and AF is not mediated through these known risk factors for AF.

We have previously reported the results of our genome-wide association study of stroke where the AF variants on chromosome 4q25 were found to significantly associate with IS, and as expected, with the strongest risk for the CES subclass of IS[4]. To assess the correlation between rs7193343 and stroke, we tested this variant in five IS case-control sample sets of European

descent, from Iceland, West-Germany, South-Germany, Sweden and the United Kingdom. Combined analysis of the five datasets showed significant association between rs7193343 and IS (OR=1.11, 95% CI: 1.04, 1.17, *P*=0.00054) (Table 2). Association analysis of IS subclasses showed significant association between rs7193343 and CES with an OR comparable to the association between rs7193343 and AF (OR=1.22, 95% CI: 1.10, 1.35, *P*=0.00021) (Table 2).

The sequence variant rs7193343 is an intronic SNP located in the zinc finger homeobox 3 (*ZFHX3*) gene on chromosome 16q22, also called AT motif-binding factor 1 (*ATBF1*). This gene encodes a transcription factor named Atbf1 which was first described as an enhancer of the human alpha-fetoprotein (*AFP*) gene expression in the liver[5]. At the time of its discovery it was the largest DNA binding protein reported and the first protein shown to contain multiple homeodomains and multiple zinc finger motifs[5]. The gene has since been associated with regulation of growth and differentiation of several tissues, including neuronal and skeletal muscle differentiation[6]. We did not observe an association of rs7193343 with the expression of *ZFHX3* in blood or adipose tissue (Supplementary Table 3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Association of rs7193343-T on chromosome 16q22 with AF/AFl. Results are shown for the Icelandic discovery data set, an Icelandic follow-up dataset, the two Icelandic datasets combined, and follow-up datasets from Norway, and the US, and for all the datasets combined using a Mantel-Haenzel model. Shown are the number of cases and controls for each study group, the frequency in cases and in controls (in parenthesis), the OR with 95% confidence intervals (CI), and *P* values assuming the multiplicative model. For the Icelandic study groups, the *P* values and CI were adjusted for relatedness as described in the method section.

Sample (cases/controls)	rs7193343 T Frequency	OR (95% CI)	P
Iceland			
Discovery (2,381/33,723)	0.229 (0.199)	1.20 (1.11, 1.29)	$3.1 \cdot 10^{-6}$
Follow-up (970/1,939)	0.238 (0.205)	1.21 (1.05, 1.39)	0.0065
Combined (3,352/35,662)	0.231 (0.199)	1.21 (1.13, 1.29)	$9.2 \cdot 10^{-9}$
Other European ancestry			
Norway (722/711)	0.177 (0.166)	1.08 (0.89, 1.31)	0.45
US (735/729)	0.183 (0.139)	1.39 (1.14, 1.70)	0.0010
Combined (1,457/1,440)	- (-)	1.22 (1.06, 1.40)	0.0046
All European ancestry			
Combined (4,809/37,102)	- (-)	1.21 (1.14, 1.29)	$1.4 \cdot 10^{-10}$
Chinese ancestry			
Hong Kong (285/2,763)	0.686 (0.676)	1.05 (0.87, 1.26)	0.63

Table 2

Association of rs7193343-T on chromosome 16q22 with ischemic stroke and cardioembolic stroke. Results are shown for datasets for Iceland, Sweden, South-Germany, West-Germany and the UK, and for all the datasets combined using a Mantel-Haenzel model. Shown are the number of controls and number of cases with each phenotype, the frequency in controls and in cases, the OR with 95% confidence intervals (CI), and *P* values assuming the multiplicative model. For the Icelandic study group, the *P* values and CI were adjusted for relatedness as described in the method section.

	Controls		**Ischemic stroke**				**Cardioembolic stroke**			
Sample	**N**	**Freq**	**N**	**Freq**	**OR (95% CI)**	**P**	**N**	**Freq**	**OR (95% CI)**	**P**
Iceland	36,430	0.201	2,308	0.208	1.05 (0.97, 1.13)	0.22	419	0.223	1.16 (0.98, 1.37)	0.084
Sweden	700	0.156	856	0.183	1.21 (1.00, 1.46)	0.046	151	0.172	1.12 (0.80, 1.57)	0.50
South-Germany	1,088	0.167	1,133	0.187	1.14 (0.98, 1.33)	0.090	283	0.214	1.35 (1.07, 1.71)	0.011
West -Germany	1,107	0.164	1,353	0.187	1.17 (1.01, 1.36)	0.034	540	0.192	1.21 (1.00, 1.47)	0.046
UK	573	0.123	585	0.152	1.28 (1.01, 1.62)	0.042	62	0.161	1.37 (0.81, 2.32)	0.24
Combined	39,898	-	6,235	-	1.11 (1.04, 1.17)	0.00054	1,454	-	1.22 (1.10, 1.35)	0.00021