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Atrial fibrillation associated genetic variation near *PITX2* gene increases the risk of preeclampsia

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ABSTRACT

Objectives: SNP rs2200733 located near *PITX2* gene is associated with the risk of atrial fibrillation. Preeclamptic women are at increased risk of developing cardiovascular disease like atrial fibrillation. Whether this translates into an association between SNP rs2200733 and preeclampsia is not known. Therefore, we determined the association of SNP rs2200733 (C/T) with the risk of preeclampsia.

Study design: A hospital based prospective case-control study involving 585 pregnant women of whom 285 were preeclamptic and 300 were normotensive. SNP rs2200733 was genotyped by PCR-RFLP method.

Main outcome measures: Statistical significance of the difference in the minor allele frequency between case and control groups was determined by Fisher's exact test.

Results: Minor allele frequency was 21.4% among preeclamptic pregnant women and 13.7% among normotensive pregnant women ($P = 0.00064$; odds ratio = 1.72 (0.95 CI: 1.23–2.41). The measures of association were heterogeneous when compared after categorisation of the preeclamptic group into clinical sub-groups. The association was not significant with the eclampsia sub-group ($P = 0.39$) but relatively higher with the sub-group not superimposed by eclampsia ($P = 0.0000048$; odds ratio = 2.10 [0.95CI: 1.50–2.92]). Furthermore, the association was relatively higher with the sub-group involving intrauterine growth retardation and intrauterine death ($P = 0.00017$; odds ratio = 2.89 (0.95CI: 1.65–4.94)].

Conclusions: Minor allele of SNP rs2200733 is associated with the risk of preeclampsia. SNP rs220073 may represent a common risk factor that predispose women to develop both preeclampsia during pregnancy and cardiovascular disease later on.

1. Introduction

Preeclampsia (PE) is a multifactorial condition with a genetic component. Contribution of genetic factors to the development of PE is supported by twin and familial aggregation studies. Heritability of PE is estimated to be about 0.54 [1]. An expanding body of evidence shows that women who develop PE are at increased risk of cardiovascular disease (CVD) and cerebrovascular events 10–15 years later in their life [2,3]. A *meta*-analysis of 43 studies found that women with a history of pre-eclampsia are at significantly increased odds of fatal or diagnosed CVD (odds ratio = 2.28) and cerebrovascular disease (odds ratio = 1.76) [4]. In addition, the children borne by women who develop PE are at two-fold higher risk of stroke in the adulthood [5]. At present, the mechanistic basis for the link between PE and CVD is not clear. PE either induces physiological events that later manifest as CVD or the two disorders share common risk factors. Epidemiological evidence point towards shared risk factors as the putative link between the conditions. For instance, risk factors such as obesity, hyperlipidaemia, hypertension, and insulin resistance, are shared by PE as well as CVD [6,7]. Furthermore, PE and CVD share a common mode of pathogenesis in the involvement of endothelial dysfunction and inflammation [8]. The presence of common risk factors have led to the hypothesis that maternal constitution, in the form of predisposition to vascular and metabolic disease, forms the basis for both PE and the subsequent risk of CVD rather than PE as the direct cause for subsequent onset of CVD [9]. The shared risk factor hypothesis has encouraged the search for genetic determinants common to both CVD and PE. Previous studies have noticed that CVD associated genetic variants occur at

Abbreviations: CI, Confidence Interval; CVD, Cardio-Vascular Disease; HELLP, Hemolysis Elevated Liver Low Platelet Count; IUD, Intrauterine Death; IUGR, Intra-Uterine Growth Restriction; OR, Odds Ratio; PCR-RFLP, Polymerase Chain Reaction – Restriction Fragment Length Polymorphism; PE, Preeclampsia; SNP, Single Nucleotide Polymorphism

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comparatively higher frequency in pre-eclamptic women [10,11]. Some of genetic variants shared by CVD and PE are rs2322659 in lactase gene (*LCT*), rs35821928 in low density lipoprotein receptor-related protein 1B gene (*LRP1B*), rs115015150 in rho family GTPase 3 gene (*RND3*) and rs17783344 in grancalcin gene (*GCA*) [10,11].

Large population based studies have reported up to 61% relative increase in the risk of CVD in women with a history of placental syndrome [12]. The common CVDs observed in these women are, in the order of incidence, heart failure, atrial dysrhythmia and ventricular dysrhythmia. The epidemiological connection between PE and atrial dysrhythmia encouraged us to search for genetic variants that may be shared by the two conditions. We found that the single nucleotide polymorphism (SNP), rs2200733, is well established with the risk of developing atrial fibrillation and ischemic stroke [13–15]. SNP rs2200733 is located 150 kb upstream of *PITX2* (paired-like homeodomain 2) gene that codes for a transcription factor. Atrial fibrillation is one of the common forms of dysrhythmias seen in clinical practice [16]. Therefore, we formulated the hypothesis that SNP rs2200733 may be associated with the risk of developing PE and undertook this study to test it.

2. Material and methods

2.1. Study design

A prospective case-control design was adopted for the study. A total of 285 preeclamptic and 300 normotensive pregnant women were enrolled in the study. Participants were enrolled from the Department of Obstetrics and Gynaecology, R. L. Jalappa Hospital and Research Centre, Kolar, Karnataka, India. The study was approved by the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Kolar, India. Informed consent was obtained from each participant before enrolment. PE was diagnosed in pregnant women following the criteria of The American College of Obstetricians and Gynaecologists [17]. Normotensive pregnant women who had no complication till delivery and no history of hypertension were included as control. SNP rs2200733 was genotyped in both the groups and subjected to statistical analysis to measure its association with PE.

2.2. Patient selection

Women were diagnosed with preeclampsia on the basis of the following criteria: (i) new onset hypertension (two readings of systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg measured 4 h apart while the patient is on bed rest (ii) ≥20 weeks of gestation (iii) new onset proteinuria (> 300 mg protein for 24 h of urine or +1 on dipstick) (iv) in the absence of proteinuria, other symptoms like Hemolysis Elevated Liver Low Platelet syndrome, edema, thrombocytopenia, impaired liver function, new-onset cerebral or visual disturbances and renal insufficiency (in the absence of other renal disease) nausea, severe headache and convulsions [17]. Inclusion criteria were: (i) pregnant women with preeclampsia, (ii) superimposed eclampsia, (iii) singleton and multiple gestation and, (iv) primigravida and multigravida condition. Exclusion criteria were: (i) pregnant women with chronic hypertension and (ii) co-morbidities such as diabetes mellitus, epilepsy, respiratory diseases, and heart diseases.

2.3. Genotyping SNP rs2200733

Blood sample leftover from routine haematology analysis was used to isolate genomic DNA by salting out method [18]. DNA purity was determined by UV spectrophotometry (Perkin Elmer model Lambda 35, Waltham, USA). PCR was set-up with the primers: 5′ AGT AAT TCT GCC TTG GTG GTA CTT G 3′ and 5′CGG TTA GAA TCT CAC ACT GTG AAT G 3′. 20 µl reaction mixture included 1X assay buffer, 100 ng genomic DNA, 0.2 mM dNTP, 10 pmol of each primer, 1.5 mM $MgCl_2$ and 1 unit *Taq* DNA polymerase (Bangalore Genei, Bengaluru, India). The program comprised of an initial denaturation at 95 °C for 3 mins followed by 40 cycles at 95 °C for 30 sec, 65 °C for 30 sec and 72 °C for 1 min; final extension involved 10 mins at 72 °C. The PCR product was analysed on 2% agarose gel. The 187 bp amplicon was subjected to restriction digestion with 5 units of BclI (New England BioLabs, Ipswich, USA) at 50 °C for 16 h and analysed on 3% agarose gel with ethidium bromide staining (Supplemental Fig. 1). T allele was visible as an uncut 187 bp fragment while the C allele is cleaved to produce a 150 and 37 bp fragments. The 37 bp fragment was not visible on the gel due to its small size; however, this does not affect genotype calling.

2.4. Statistical analysis

Statistical analysis was done using the Openepi web-tool [19]. Differences in allele frequencies and genotype distribution between groups were compared by calculating *P*-value from Fisher's exact test. Additive genetic model was tested by means of extended Mantel Haenszel Chi Square for linear trend with a *P*-value for one degree of freedom. *P*-value less than 0.05 was considered as significant. The study population was tested for conformity to Hardy-Weinberg Equilibrium using the web-tool 'Simple Hardy-Weinberg Calculator' [20].

3. Results

Clinical parameters of the study groups are given in Table 1. Eclampsia was the most common co-morbidity seen among the patients, followed by fetal complications in the form of intrauterine growth retardation (IUGR) or intrauterine death (IUD).

The distribution of genotypes and alleles of SNP rs2200733 among preeclamptic and normotensive pregnancies is shown in Table 2. Genotype distribution in the control group was in agreement with Hardy-Weinberg equilibrium ($\chi^2 = 3.1$). TT genotype was relatively more common among preeclamptic pregnant women (4.2%) than among normotensive pregnant women (0.7%). The difference in the distribution of genotypes between the two groups was statistically significant ($P = 0.00084$). Difference between the groups was also observed at the

Table 1
Clinical profile of the study groups.

Parameter	Preeclamptic pregnant women (n = 285)		Normotensive pregnant women (n = 300)
Age (years)	25.0 ± 3.6		24.5 ± 3.3
Gravida			
Primigravida	154 (54.03%)		121 (40.33%)
Multigravida	131 (45.96%)		179 (59.66%)
Gestation (weeks)	34.5 ± 4.0		38.0 ± 2.0
Severity			
Mild	118 (41.40%)		NA
Severe	167 (58.59%)		
Blood Pressure (mmHg)	Mild PE	Severe PE	
Systolic Blood Pressure	137.9 ± 12.0	164.2 ± 21.0	120.5 ± 10.0
Diastolic Blood Pressure	89.0 ± 9.0	111.2 ± 12.0	78.8 ± 82.0
Dipstick proteinuria			
1 +	164 (57.5%)		NA
2+	72 (25.3%)		
3+	49 (17.2%)		
Comorbidity			
Eclampsia	59 (20.7%)		NA
IUGR/IUD	38 (13.3%)		
HELLP Syndrome	8 (2.8%)		

NA: Not Applicable.

Table 2
Distribution profile of rs2200733 SNP genotypes and alleles in the study groups.

Genotype/Allele	Preeclamptic pregnant women (n = 285)	Normotensive pregnant women (n = 300)	*P*-value*
CC	175	220	0.00084
CT	98	78	
TT	12	2	
C	448	518	0.00064 [OR: 1.72 (0.95 CI: 1.25 – 2.37)]
T	122	82	

* Chi-squared test (Fisher's exact).

Table 3
Evaluation of the association between rs2200733 SNP and the risk of PE under different genetic models.

Model	Genotype	*P*-value*
Dominant	CT + TT *vs.* CC	0.0013
Recessive	TT *vs.* CT + CC	0.012
Additive	TT > CT > CC	0.0005
Over-dominant	CT *vs.* CC + TT	0.017

* Chi-squared test (Fisher's exact) for dominant, recessive and over-dominant models; Cochran-Armitage trend test for additive model.

level of allele. Frequency of the T allele was higher among preeclamptic pregnant women (21.4%) than among normotensive pregnant women (13.7%). The difference in the allele distribution between the groups was statistically significant [$P = 0.00064$; odds ratio 1.72 (0.95 CI: 1.25–2.37)]. Table 3 shows the statistical evaluation of the genotypes in dominant, recessive, additive and over-dominant genetic models. Lowest *P*-value was observed in the case of additive genetic model; odds ratio for the T allele in heterozygous and homozygous genotypes were 1.58 and 7.54 respectively.

We undertook statistical analysis after categorisation of the genetic data into clinical sub-groups. The results of the analyses are summarised in Table 4. The overall PE group was categorised with respect to the involvement of eclampsia and analysed for association. There was no significant association between SNP rs2200733 and PE superimposed by eclampsia ($P > 0.05$). The association did not change even upon considering the genotypes in the additive genetic model. In contrast, PE sub-group without superimposed eclampsia showed significant association at both genotype and allele levels; in addition, the *P*-value of this subgroup reduced by an order of 2 in comparison to that of the overall PE group. The data was also categorised with respect to severity; significant association was found with both mild and severe forms. We also categorised the overall PE group with respect to the fetal outcomes namely IUGR and IUD; significant association was observed at both genotype and allele levels and the odds ratio increased by a factor of 1.7 in comparison to the overall PE group.

Table 4
Evaluation of the association between T allele of rs2200733 SNP and clinical sub-groups of PE.

PE sub-group	Odds Ratio (0.95 CI)*	*P*-value§
$PE_{Overall}$ #	1.72 (1.23–2.41)	0.00064
$PE_{Eclampsia\ (+)}$	1.28 (0.73–2.16)	0.39
$PE_{Eclampsia\ (-)}$	2.10 (1.50–2.92)	0.0000048
$PE_{IUGR/IUD}$	2.89 (1.65–4.94)	0.00017
PE_{Mild}	1.62 (1.12–2.34)	0.0091
PE_{Severe}	1.73 (1.14–2.60)	0.0094

* Odds ratio represents comparison of the frequencies of rs2200733 SNP T allele in PE sub-groups with the frequency in normotensive pregnant women. Confidence interval is abbreviated as CI.

\# Preeclamptic Pregnant Women.

§ Chi-squared test (Fisher's exact).

4. Comment

The results of this study show that SNP rs2200733 is associated with the risk of PE. Furthermore, our results show that the association is not uniform across clinical subgroups but dependant on the involvement of eclampsia and foetal complications. Bioinformatics analysis has shown that PE represents a collection of several distinct phenotypes, with both unique and overlapping genetic contributions [21]. Also, the genes that are significantly associated with PE appear to segregate with respect to severity and co-morbidities like eclampsia and IUGR. Our observation of heterogeneity in the odds ratio across PE subgroups is in agreement with the bioinformatics analysis. The odds ratio for the risk of IUGR and IUD was 1.7 times higher compared to that for the overall PE group. Increase in the odds ratio agrees with the previous observation that the future risk of CVD is comparatively higher in the case of PE complicated by IUGR and IUD [12].

We chose SNP rs2200733 since it is well associated with the risk of atrial fibrillation and stroke [22–25]. Atrial fibrillation is the most common form of atrial dysrhythmia, a CVD that is frequently seen women with a history of PE [12,16]. Positive association between SNP rs2200733 and atrial fibrillation has been observed in Caucasian and Mongoloid populations and replicated in cohorts like Framingham Heart Study, Rotterdam Study, Vanderbilt Atrial Fibrillation Registry and German Atrial Fibrillation Network [24–28]. SNP rs2200733 is associated with stroke in the Indian population (where the current study is based) [29]. Meta-analysis comprising of 10,546 cases of atrial fibrillation and 72,789 reference individuals without atrial fibrillation has confirmed the association [$P < 0.001$; OR 1.89 (95% CI: 1.62–2.16] [22].

SNP rs2200733 is located 150 kb upstream of *PITX2* gene at chromosomal locus 4q25. *PITX2* gene plays an important role in embryonic development particularly in the development of left–right symmetry of the heart [30]. Expression of *PITX2* gene has been observed in human and mouse left atria [31]. Deletion of *PITX2* gene in mice results in the development of atrial arrhythmia upon programmed stimulation and also reduced expression of cardiac sodium and potassium channels [32,33]. The potential involvement of *PITX2* gene variants in the risk of atrial fibrillation is supported by the observation of strong association in a *meta*-analysis of several GWAS studies [34]. Reduced expression of *PITX2* gene in patients with sustained atrial fibrillation is considered as an evidence for a potential molecular link between *PITX2* gene loss-of-function and atrial fibrillation [35].

In addition to the myocardium, expression of PITX2 protein has also been observed in the human placenta [36,37]. However, the role of PITX2 protein in the placenta is not clear. *PITX2* gene is involved in the cross-regulation of another CVD associated gene *viz.*, *ZFHX3* [38]. Both PITX2 and ZFHX3 proteins are involved in the regulation of *NPPA* gene, which codes for atrial natriuretic peptide, a cardiac hormone with vaso-regulatory function [38]. Interestingly, *NPPA* gene is expressed in the placenta in addition to atrial myocytes [39]. Furthermore, reduction in the levels of placenta-derived atrial natriuretic peptide has been shown to play a role in the impairment of spiral artery remodelling, a cardinal pathophysiological event seen in PE.

Replication studies in other ethnic populations and their *meta*-

analysisis necessary to evaluate whether the association observed in this study is a population specific or global phenomenon. Furthermore, evaluation of PITX2 protein expression in the placental tissue from preeclamptic and normotensive pregnant women is warranted in order to understand the impact of SNP rs2200733 at the level of gene regulation.

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6. Disclosure of interests

UR, MM and SB are listed as inventors in patent application number 2017410442925 filed in India

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.preghy.2018.06.023.

References

[1] R.H. Salonen, P. Lichtenstein, L. Lipworth, et al., Genetic effects on the liability of developing pre-eclampsia and gestational hypertension, Am. J. Med. Genet. 91 (2000) 256–260.
[2] L. Bellamy, J.P. Casas, A.D. Hingorani, et al., Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis, BMJ 335 (2007) 974.
[3] E. Kajantie, J.G. Eriksson, C. Osmond, et al., Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study, Stroke 40 (2009) 1176–1180.
[4] M.C. Brown, K.E. Best, M.S. Pearce, et al., Cardiovascular disease risk in women with pre-eclampsia: systematic review and meta-analysis, Eur. J. Epidemiol. 28 (2013) 1–19.
[5] E.F. Davis, A.J. Lewandowski, C. Aye, et al., Clinical cardiovascular risk during young adulthood in offspring of hypertensive pregnancies: insights from a 20-year prospective follow-up birth cohort, BMJ Open. 5 (2015) e008136.
[6] A.L. Berends, C.J. de Groot, E.J. Sijbrands, et al., Shared constitutional risks for maternal vascular-related pregnancy complications and future cardiovascular disease, Hypertension 51 (2008) 1034–1041.
[7] E.B. Magnussen, L.J. Vatten, T.I. Lund-Nilsen, et al., Pre pregnancy cardiovascular risk factors as predictors of pre-eclampsia: population based cohort study, BMJ 335 (2007) 978.
[8] I. Craici, S. Wagner, V.D. Garovic, Preeclampsia and future cardiovascular risk: formal risk factor or failed stress test? Ther. Adv. Cardiovasc. Dis. 2 (2008) 249–259.
[9] N. Sattar, I.A. Greer, Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? BMJ 325 (2002) 157–160.
[10] M. Loset, M.P. Johnson, P.E. Melton, et al., Preeclampsia and cardiovascular disease share genetic risk factors on chromosome 2q22, Pregnancy Hypertens. 4 (2014) 178–185.
[11] M.P. Johnson, S.P. Brennecke, C.E. East, et al., Genetic dissection of the pre-eclampsia susceptibility locus on chromosome 2q22 reveals sharednovel risk factors for cardiovascular disease, Mol. Hum. Reprod. 19 (2013) 423–437.
[12] J.G. Ray, M.J. Schull, J.C. Kingdom, et al., Heart failure and dysrhythmias after maternal placental syndromes: HAD MPS Study, Heart. 98 (2012) 1136–1141.
[13] M.S. Olesen, M.W. Nielsen, S. Haunso, et al., Atrial fibrillation: the role of common and rare genetic variants, Eur. J. Hum. Genet. 22 (2014) 297–306.
[14] S. Gretarsdottir, G. Thorleifsson, A. Manolescu, et al., Risk variants for atrial fibrillation on chromosome 4q25 associate with ischemic stroke, Ann. Neurol. 64 (2008) 402–409.
[15] Y.Y. Cao, F. Ma, Y. Wang, et al., rs2200733 and rs10033464 on chromosome 4q25 confer risk of cardio embolic stroke: an updated meta-analysis, Mol. Biol. Rep. 40 (2013) 5977–5985.
[16] C.R.C. Wyndham, Atrial fibrillation: the most common arrhythmia, Tex Heart Inst J. 27 (2000) 257–267.
[17] Hypertension in Pregnancy. Report of the American College of Obstetricians and Gynaecologists (ACOG) Task Force on Hypertension in Pregnancy 2013; ISBN 978-1-934984-28-4.
[18] S.A. Miller, D.D. Dykes, H.F. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells, Nucl. Acids Res. 16 (1988) 1215.
[19] A.G. Dean, K.M. Sullivan, M.M. Soe. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. www.OpenEpi.com, updated 2013/04/06, accessed 2017/12/01.
[20] S. Rodriguez, T.R. Gaunt, I.N. Day, Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies, Am. J. Epidemiol. 169 (2009) 505–514.
[21] E.W. Triche, A. Uzun, A.T. DeWan, et al., Bioinformatic approach to the genetics of preeclampsia, Obstet. Gynecol. 123 (2014) 1155–1161.
[22] S. Mohanty, P. Santangeli, R. Bai, et al., Variant rs2200733 on chromosome 4q25 confers increased risk of atrial fibrillation: evidence from a meta-analysis, J. Cardiovasc Electrophysiol. 24 (2013) 155–561.
[23] D.F. Gudbjartsson, D.O. Arnar, A. Helgadottir, et al., Variants conferring risk of atrial fibrillation on chromosome 4q25, Nature 448 (2007) 353–357.
[24] M. Wnuk, J. Pera, J. Jagiełła, et al., The rs2200733 variant on chromosome 4q25 is a risk factor for cardioembolic stroke related to atrial fibrillation in Polish patients, Neurol. Neurochir. Pol. 45 (2011) 148–152.
[25] L. Shi, C. Li, C. Wang, et al., Assessment of association of rs2200733 on chromosome 4q25 with atrial fibrillation and ischemic stroke in a Chinese Han population, Hum. Genet. 126 (2009) 843–849.
[26] C. Viviani Anselmi, V. Novelli, R. Roncarati, et al., Association of rs2200733 at 4q25 with atrial flutter/fibrillation diseases in an Italian population, Heart. 94 (2008) 1394–1396.
[27] Y. Mints, H. Yarmohammadi, I.M. Khurram, et al., Association of common variations on chromosome 4q25 and left atrial volume in patients with atrial fibrillation, Clin. Med. Insights Cardiol. 9 (2015) 39–45.
[28] S. Kaab, D. Darbar, C. van Noord, et al., Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation, Eur. Heart J. 30 (2009) 813–819.
[29] A. Bhanushali, A. Nair, G. Jagdale, et al., Association of genetic variants at the 4q25 locus with atrial fibrillation in indian population, J. Clin. Lab. Anal. 31 (2017) 12–19.
[30] D. Franco, M. Campione, The role of Pitx2 during cardiac development. Linking left-right signaling and congenital heart diseases, Trends Cardiovasc Med. 13 (2003) 157–163.
[31] P.C. Kahr, U. Piccini, L. Fabritz, et al., Systematic analysis of gene expression differences between left and right atria in different mouse strains and in human atrial tissue, PLoS One 6 (2011) 26389.
[32] P. Kirchhof, P.C. Kahr, S. Kaese, et al., PITX2c is expressed in the adult left atrium, and reducing Pitx2c expression promotes atrial fibrillation inducibility and complex changes in gene expression, Circ Cardiovasc Genet. 4 (2011) 123–133.
[33] Y. Tao, M. Zhang, L. Li, et al., Pitx2, an atrial fibrillation predisposition gene, directly regulates ion transport and intercalated disc genes, Circ Cardiovasc Genet. 7 (2014) 23–32.
[34] P.T. Ellinor, K.L. Lunetta, C.M. Albert, et al., Meta-analysis identifies six new susceptibility loci for atrial fibrillation, Nat Genet. 44 (2012) 670–675.
[35] A. Chinchilla, H. Daimi, E. Lozano-Velasco, et al., PITX2 insufficiency leads to atrial electrical and structural remodeling linked to arrhythmogenesis, Circ Cardiovasc Genet. 4 (2011) 269–279.
[36] https://www.proteinatlas.org/ (Accessed 27 Nov 2017).
[37] L. Fagerberg, B.M. Hallström, P. Oksvold, et al., Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics, Mol. Cell Proteomics. 13 (2014) 397–406.
[38] Y. Huang, C. Wang, Y. Yao, et al., Molecular Basis of gene-gene interaction: cyclic cross-regulation of gene expression and post-GWAS gene-gene interaction involved in atrial fibrillation, PLoS Genet 11 (2015) e1005393.
[39] Y. Cui, W. Wang, N. Dong, et al., Role of corin in trophoblast invasion and uterine spiral artery remodeling in pregnancy, Nature 484 (2012) 246–250.