SHORT REPORT/*KRÓTKIE DONIESIENIE*

The rs2200733 variant on chromosome 4q25 is a risk factor for cardioembolic stroke related to atrial fibrillation in Polish patients

Polimorfizm rs2200733 na chromosomie 4q25 jest czynnikiem ryzyka udaru sercowozatorowego związanego z migotaniem przedsionków w populacji polskiej

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Neurologia i Neurochirurgia Polska 2011; 45, 2: 148–151

Abstract

Background and purpose: A few single nucleotide polymorphisms (SNPs) on chromosome 4q25, associated with atrial fibrillation (AF), are risk factors for ischaemic stroke. We studied the significance of the SNP rs2200733 on chromosome 4q25 in different types of cardioembolic (CE) stroke.
Material and methods: We genotyped 428 controls and 301 CE stroke patients, among whom 197 (65.4%) presented with high risk sources of embolism (CE stroke related to AF) and 104 with medium risk sources (CE stroke unrelated to AF). The SNP rs2200733 was analysed using real-time polymorphism chain reaction.
Results: Both univariate and multivariate regression analyses showed that the studied variant affected risk of all CE strokes or CE strokes related to AF in recessive and additive models. The two types of CE stroke differed significantly in demographics and distribution of vascular risk factors.
Conclusions: The SNP rs2200733 on chromosome 4q25 is a risk factor for CE stroke related to AF only.

Key words: cardioembolic stroke, atrial fibrillation, polymorphism.

Streszczenie

Wstęp i cel pracy: Kilka polimorfizmów na chromosomie 4q25, związanych z migotaniem przedsionków, jest czynnikami ryzyka udaru niedokrwiennego mózgu. Przeanalizowano znaczenie polimorfizmu rs2200733 na chromosomie 4q25 w różnych typach udaru sercowozatorowego.
Materiał i metody: Badany polimorfizm oznaczono u 428 osób tworzących grupę kontrolną oraz u 301 chorych na udar sercowozatorowy, spośród których 197 (65,4%) miało źródło zatorowości o dużym ryzyku (udar sercowozatorowy związany z migotaniem przedsionków), a 104 o pośrednim ryzyku (udar sercowozatorowy niezwiązany z migotaniem przedsionków). Do analizy polimorfizmu rs2200733 wykorzystano reakcję łańcuchową polimerazy DNA z analizą ilości produktu w czasie rzeczywistym.
Wyniki: Zarówno jedno-, jak i wieloczynnikowa analiza regresji logistycznej wykazały, że badany wariant wpływał na ryzyko wystąpienia wszystkich udarów sercowozatorowych oraz tych związanych z migotaniem przedsionków w modelach recesywnym i addytywnym. Dwa typy udaru sercowozatorowego różniły się w zakresie czynników demograficznych oraz rozkładu naczyniowych czynników ryzyka.

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Received: 25.08.2010; accepted: 19.02.2011
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Wnioski: Polimorfizm rs2200733 na chromosomie 4q25 jest czynnikiem ryzyka jedynie udaru sercowozatorowego związanego z migotaniem przedsionków.

Słowa kluczowe: udar sercowozatorowy, migotanie przedsionków, polimorfizm.

Introduction

A recent genome-wide association study (GWAS) revealed that a haplotype block on chromosome 4q25 predisposed to atrial fibrillation (AF) [1]. In this block, two variants were in strong linkage disequilibrium and defined three haplotypes. One of them, rs2200733, increased the risk of AF by 1.72, and the second one, rs10033464, by 1.39. The association of the rs2200733 variant with AF was then confirmed in several replication populations [2].

The significance of the rs2200733 variant as a risk factor for stroke was studied in another large GWAS [3]. The results from this study, combined with the data from four replication European populations, showed that the T allele of the rs2200733 variant reached GWAS significance as a risk factor for all strokes without considering aetiology, cardioembolic (CE) or non-CE strokes [3].

In the previous study performed in co-operation with investigators from 5 different countries and including 4190 ischaemic stroke patients and 3740 controls, we replicated the association of the single nucleotide polymorphism (SNP) rs2200733 with atrial fibrillation and ischaemic stroke of cardioembolic aetiology [4]. In the present case-control study, we evaluated the role of the rs2200733 variant on chromosome 4q25 in patients with cardioembolic stroke related or unrelated to AF. So far such division of cardioembolic stroke patients has never been performed according to the genetic risk factors.

Material and methods

Consecutive patients with CE stroke, diagnosed according to the TOAST criteria [5], admitted to the Stroke Unit, Department of Neurology, University Hospital in Cracow, between 2005 and 2010, were included in the study.

Information on potential cardiac sources of embolism was based on previous medical documentation or investigations performed during the current hospitalization (ECG – 100%, carotid ultrasound – 71%, echocardiography – 62%). There were 197 cases with AF (65.4%). In the remaining 104 cases, CE stroke was diagnosed because of low ejection fraction due to ischaemic heart disease ($n = 72$), myocardial infarction ($n = 13$), atrial septal defect ($n = 12$) or bioprosthetic cardiac valve ($n = 7$) (CE stroke unrelated to AF).

Controls were collected as described previously [6]. Information on demographics and vascular risk factors from patients and controls was collected as described previously [6]. The rs2200733 polymorphism was identified using real-time polymorphism chain reaction RT-PCR [7]. This study was performed according to the Helsinki Declaration with approval of the local Ethical Committee. All individuals gave informed consent.

Comparisons between the groups were made with χ^2 test or Student's t-test. The Hardy-Weinberg equilibrium was tested by the χ^2 method. The association of the rs2200733 genotypes with the risk of CE stroke was tested using univariate and multivariate logistic regression analyses (SPSS 10 for Windows).

Results

The study included 301 CE stroke patients and 428 controls. There was no deviation from Hardy-Weinberg equilibrium regarding the SNP rs2200733 in the studied groups.

Patients with CE stroke related to AF as compared to others were older, more often male and had different distribution of the vascular risk factors (Table 1).

All CE stroke patients had significantly higher prevalence of the genotypes with the T allele (CC – 189 [62.8%], CT – 101 [33.6%], TT – 11 [3.7%]) than controls (CC – 307 [71.7%], CT – 109 [25.5%], TT – 12 [2.8%]) ($\chi^2, p < 0.05$). The distribution of the polymorphism was significantly different between CE strokes with AF (CC – 116 [58.9%], CT – 70 [35.5%], TT – 11 [5.6%]) and without AF (CC – 73 [60.2%], CT – 31 [29.8%], TT – 0) ($\chi^2, p < 0.05$).

Both univariate and multivariate regression analyses revealed that the T allele of the studied variant af-

Table 1. The profile of risk factors in patients with cardioembolic stroke related and unrelated to atrial fibrillation

	Cardioembolic stroke related to atrial fibrillation (N = 197)	Cardioembolic stroke unrelated to atrial fibrillation (N = 104)	OR (95% CI)*
Hypertension	168 (85.3%)	75 (72.1%)	2.24 (1.25-4.01)**
Diabetes mellitus	69 (35%)	20 (19.2%)	2.26 (1.28-4.00)**
Ischaemic heart disease	147 (74.6%)	67 (64.4%)	1.62 (0.97-2.71)
Myocardial infarction	35 (17.8%)	46 (44.2%)	0.28 (0.17-0.49)**
Hypercholesterolaemia	79 (40.1%)	64 (61.5%)	0.41 (0.25-0.67)**
Smoking***	23 (11.7%)	41 (39.4%)	0.19 (0.11-0.35)**

**OR (95% CI); logistic regression analysis adjusted for age and gender*

***$p < 0.05$*

****26 (13.2%) patients with cardioembolic stroke related to atrial fibrillation and 12 (11.5%) unrelated to atrial fibrillation did not give information on their smoking habits*

Table 2. Relative risk of cardioembolic (CE) ischaemic stroke in carriers of the SNP rs2200733

	Crude OR*	*p*-value	OR adjusted for all studied risk factors**	*p*-value	OR adjusted for all studied risk factors** except for AF	*p*-value
All CE strokes						
dominant model	1.31 (0.57-3.02)	0.52	0.61 (0.17-2.27)	0.47	1.53 (0.49-4.80)	0.47
recessive model	**1.50 (1.10-2.06)**	**0.01**	1.37 (0.83-2.67)	0.22	**1.64 (1.06-2.53)**	**0.025**
additive model	**1.40 (1.06-1.83)**	**0.01**	1.20 (0.78-1.87)	0.41	**1.51 (1.04-2.21)**	**0.03**
CE strokes related to AF						
dominant model	2.05 (0.89-4.74)	0.09	N/A	–	2.74 (0.78-9.65)	0.11
recessive model	**1.77 (1.24-2.52)**	**0.002**	N/A	–	**2.14 (1.27-3.62)**	**0.004**
additive model	**1.64 (1.22-2.21)**	**0.001**	N/A	–	**1.95 (1.25-3.04)**	**0.003**
CE strokes unrelated to AF						
dominant model	N/A	–	N/A	–	N/A	–
recessive model	1.08 (0.67-1.73)	0.76	N/A	–	1.24 (0.67-2.31)	0.50
additive model	0.95 (0.62-1.46)	0.82	N/A	–	1.07 (0.61-1.89)	0.80

**Odds ratios (OR) with 95% confidence intervals*

***Age, gender, hypertension, diabetes mellitus, ischaemic heart disease, myocardial infarction, hypercholesterolaemia, smoking and atrial fibrillation (AF)*

N/A – non-applicable (due to the lack of TT genotype in CE strokes unrelated to AF)

fected the risk of all CE strokes or CE strokes related to AF in recessive and additive models of inheritance (Table 2).

Discussion

Based on our data, we suggest that patients with the two types of CE stroke, with high or medium risk sources of cardioembolism, should be considered separately because they present with different distribution of vascular and genetic risk factors. In the present study it was also revealed that patients with CE stroke related or unrelated to AF differed in terms of demographic presentation. To our knowledge, this is the first study showing significant heterogeneity among CE stroke patients which, according to the TOAST criteria, belong to one subgroup. Previous studies showed only a difference in the distribution of vascular risk factors among subtypes of ischaemic stroke based on the TOAST classification [8,9].

The TOAST criteria are useful in respect of prophylaxis of ischaemic stroke. Cardioembolic stroke can be diagnosed in a wide range of cardiac disorders: from

atrial fibrillation, through recent myocardial infarction and infective endocarditis, to congestive heart failure with low ejection fraction and cardiomyopathy. All patients with these potential cardioembolic conditions should be treated with antithrombotic drugs in contrast to patients with other ischaemic stroke aetiologies in whom antiplatelet drugs represent the first line of treatment.

However, when investigating the pathophysiology and genetics of ischaemic stroke, the TOAST system seems to be outdated. Even in our research the studied polymorphism was not an independent risk factor for stroke until all CE stroke patients were divided into two subgroups, related or unrelated to AF. It is worth considering these two subtypes of CE stroke separately in future studies concerning pathophysiology and genetic risk factors of stroke.

In conclusion, we revealed that the T allele of the rs2200733 variant was a risk factor for CE stroke specifically related to AF. Although the TOAST diagnostic system in stroke genetic studies has only modest intrarater reliability [10], it is still used in the research concerning genetic risk factors for stroke. Thus, we recommend that patients with CE stroke should be divided into at least two separate subgroups on the basis of the genetic and clinical differences shown in our study. However, because of the relatively small group size, future studies are needed to confirm our results.

Conclusions

The SNP rs2200733 on chromosome 4q25 is a risk factor for CE stroke related to AF only.

Disclosure

Wojciech Turaj serves as an editor of *Neurologia i Neurochirurgia Polska*. Other authors declared no conflict of interest.

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