The **CELSR1** polymorphisms rs6007897 and rs4044210 are associated with ischaemic stroke in Chinese Han population

Yi-Hong Zhan1,2, Yi Lin1, Sui-Jun Tong2, Qi-Lin Ma2, Cong-Xia Lu2, Ling Fang1, Wei Wei1, Bin Cai1, and Ning Wang1

1Department of Neurology and Institute of Neurology, First Affiliated Hospital, Center of Neuroscience, Fujian Medical University, Fuzhou, PR China and 2Department of Neurology, The First Affiliated Hospital of Xiamen University, Xiamen, PR China

**Abstract**

**Background:** Recently, CELSR1 was identified by genome-wide association studies (GWAS) as a susceptibility gene for ischaemic stroke (IS) in Japanese individuals.

**Aim:** The goal was to examine whether CELSR1 variants are associated with IS in the Chinese Han population.

**Subjects and methods:** This study genotyped two single nucleotide polymorphisms (SNPs) of CELSR1, rs6007897 and rs4044210, in a Chinese sample of 569 IS cases and 581 controls and assessed their genotype and allele associations with IS.

**Results:** The results showed that rs6007897 and rs4044210 variants of CELSR1 were significantly \( p < 0.01 \) associated with IS. These associations remained after adjustment for age, gender, smoking status, hypertension, diabetes mellitus and hypercholesterolemia. In addition, a significant association was observed of rs6007897 and rs4044210 of CELSR1 with large artery atherosclerosis (LAA), a sub-type of IS \( p < 0.01 \).

**Conclusion:** Taken together, the present study has proven for the first time that CELSR1 is a susceptibility gene for IS in the Chinese Han population, especially for LAA.

**Keywords**

CELSR1, Chinese Han, ischaemic stroke, LAA, single nucleotide polymorphism

**History**

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**Introduction**

Ischaemic stroke (IS) is one of the leading causes of death and disability worldwide (Smith et al., 2009). It is a complex disease resulting from the interaction of many genetic and environmental factors (Domingues-Montanari et al., 2008). Substantial evidence for a genetic contribution to IS has been provided by extensive studies in twins, families and animal models (Hassan & Markus, 2000; Tournier-Lasserve, 2002), but the genes which act as risk factors for IS in the general population remain undetermined. Genome-wide association studies (GWAS) have previously uncovered unsuspected common gene variants underlying the risk of complex diseases such as diabetes and coronary disease (McPherson et al., 2007; Scott et al., 2007).

Recently, Yamada et al. (2009) identified Cadherin, epidermal growth factor (EGF) laminin A G-type repeats (LAG) seven-pass G-type receptor 1 (CELSR1) as a susceptibility gene for ischaemic stroke in Japanese individuals using GWAS. The association of rs6007897 (A → G, Thr2268Ala) and rs4044210 (A → G, Ile2107Val) of CELSR1 with IS risk were further validated in an independent Portuguese sample (Gouveia et al., 2011). However, due to the large amount of data produced, it is difficult to distinguish between true and false positive variants, even with the availability of potent statistical tools (Pattin & Moore, 2008). In addition, different ethnic groups have different genetic backgrounds and environment pressures, which represent confounding factors in genetic association studies. Therefore, more replicated studies to confirm the results of GWAS are necessary. Hence, we performed a case-control study to identify whether rs6007897 and rs4044210 variants of CELSR1 are associated with IS in the Chinese Han population.

**Subjects and methods**

**Subjects**

IS cases \( (n = 569) \) and controls \( (n = 581) \) were included in the present study. All subjects enrolled in the study were from a Chinese Han population in South China who visited or were admitted to the First Affiliated Hospital of Fujian Medical University between 1 September 2009 and 25 September 2013. Informed consent was obtained and the study was approved by the research committee and the institutional review board of the First Affiliated Hospital of Fujian Medical University. Detailed medical history and demographic information were obtained by questionnaire. Cerebral infarction was diagnosed when a new and abrupt focal neurological deficit occurred which was accompanied by neurological symptoms and when signs persisted for over
24 hours. Positive findings were confirmed by computed tomography or magnetic resonance imaging (or both) of the head. Cases were classified into the following sub-types using a modified Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification (Adams et al., 1993): large artery atherosclerosis (LAA); small artery occlusion (SAO); cardi- oembolism; stroke of other determined aetiology and stroke of undetermined aetiology. Only patients with LAA and SAO sub-types were included for further analysis. Among them, 422 patients had LAA sub-type and 147 patients had SAO sub-type. Individuals who visited outpatient clinics of the participating hospital for consecutive annual health check-ups were recruited as control subjects. Detailed questionnaires were obtained to evaluate the absence of stroke history and to confirm the absence of neurological deficit history. They either had or did not have conventional risk factors for stroke, including hypertension (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or administration of anti-hypertensive medication), diabetes mellitus (concentration of fasting blood glucose ≥7.00 mmol/L, content of haemoglobin A1c ≥6.5% or administration of anti-diabetic medication) or hypercholesterolemia (concentration of serum total cholesterol ≥5.72 mmol/L or administration of lipid-lowering medication). These subjects also had no history of the following diseases: ischaemic or haemorrhagic stroke or other cerebral diseases, coronary heart disease, peripheral arterial occlusive disease or other atherosclerotic diseases; or other thrombotic, embolic or haemorrhagic disorders.

Genotyping of single nucleotide polymorphisms (SNPs)

Genomic DNA was extracted from peripheral blood with a QIAamp DNA Blood Minikit (QIAGEN GmbH, Hilden, Germany). Genotyping was performed by PCR-RFLP analysis by using the following primers: rs6007897 (forward: 5'-ATG GCC AGC CAG CGT CAG TG-3'; reverse: 5'-TGA AGA AGT CGG CTG GGA AG-3'; 0); and rs4044210 (forward: 5'-ACT CTC TCA CGC ATA CAC GAA CAC-3'; reverse: 5'-CAG TTT GAG TTT CAG GCT TTG CAC-3'; 0). PCR products were digested with BsaHI for rs6007897 and XcmI for rs4044210 according to the manufacturer’s recommendations (New England Biolabs, Beverly, MA), followed by a 2.5% agarose gel electrophoresis. To confirm the genotypes of the two SNPs, DNA sequencing was performed with an ABI Prism 3730 genetic analyser (Applied Biosystems Inc., Foster City, CA) by using an ABI dye terminator cycle sequencing kit (Applied Biosystems Inc.).

Statistical analysis

Comparisons of continuous variables were tested by Student’s t-test. Allelic and genotypic frequencies were compared between IS subjects and controls using a chi-square test. The deviation from Hardy–Weinberg expectation for the variants was tested by a chi-square test. Multivariable logistic regression analysis was performed with ischaemic stroke as a dependent variable and independent variables that included age, sex (1 = man, 2 = woman), smoking status (0 = non-smoker, 1 = smoker), drink status (0 = non-drinker, 1 = drinker), diabetes mellitus, hypertension and hypercholesterolemia (0 = no history of these conditions, 1 = positive history) and genotype of each SNP. Each genotype was assessed according to dominance (0 = wild-type homozygote, 1 = heterozygote variant homozygote) and the p value, odds ratio and 95% confidence interval were calculated. Unless indicated otherwise, a p value of <0.05 was considered statistically significant. Statistical tests were performed with PASW v19.0 (IBM Corporation, Somers, NY) and LDA software v1 (Chinese National Human Genome Center, Beijing, China).

Results

Clinical characteristics of study subjects

The clinical characteristics of IS subjects and controls are summarized in Table 1. Significantly higher proportions of gender (male), smoking, hypertension, drinking, diabetes mellitus and hypercholesterolemia were observed in LAA subjects than in control subjects and only significantly higher proportions of gender (male) and hypertension were observed in SAO subjects than in control subjects.

Association of CELSR1 polymorphisms rs6007897 and rs4044210 with IS and LAA stroke sub-type

The relation of rs6007897 and rs4044210 to ischaemic stroke was examined in all subjects. All SNPs were consistent with Hardy–Weinberg expectations in both IS and control groups. The Chi-square test revealed that rs6007897 and rs4044210 of CELSR1 were significantly (p < 0.01) associated with ischaemic stroke in terms of both genotype distribution and allele frequency (Table 2). Multivariable logistic regression analysis with adjustment for age, gender, smoking status, hypertension, diabetes mellitus and hypercholesterolemia revealed that the association remained after adjustment (Table 2). Subjects with the AG genotype of rs6007897 and rs4044210 had increased risk of IS compared to those with the AA genotype (OR = 2.32, 95% CI = 1.24–4.36; OR = 2.25, 95% CI = 1.19–4.23).

In order to determine the effect of the rs6007897 and rs4044210 on the different stroke aetiologies, we also examined the association of IS sub-types LAA and SAO with the two SNPs (Table 2). We observed a significant association of rs6007897 and rs4044210 with the LAA sub-type (p < 0.01). Multivariable logistic regression adjusted for age, gender, smoking status, hypertension, diabetes mellitus and hypercholesterolemia revealed that the G-allele of rs6007897 and rs4044210 was associated with increased risk of LAA sub-type (OR = 2.84, 95% CI = 1.49–5.42; OR = 2.93, 95% CI = 1.54–5.55). However, no association of rs6007897 and rs4044210 with SAO sub-type was observed (OR = 0.66, 95% CI = 0.19–2.30; OR = 0.66, 95% CI = 0.19–2.30).

Finally, we evaluated the relation of the two SNPs to the risk of hypertension, type 2 diabetes mellitus and hypercholesterolemia (Supplementary Table 1). Comparison of genotype distributions of the rs6007897 and rs4044210 between IS subjects and controls by unadjusted and adjusted (the stratification covariate was excluded when calculating
Table 1. Clinical characteristics of control and ischaemic stroke (LAA and SAO) subjects.

| Characteristic                  | Controls | LAA subjects | Cases \( p_{\text{LAA vs. con}} \) | Controls | SAO subjects | Cases \( p_{\text{SAO vs. con}} \) |
|--------------------------------|----------|--------------|-----------------------------------|----------|--------------|-----------------------------------|
| Number of subjects            | 581      | 422          | —                                 | 147      | —            |                                    |
| Age (years)*                  | 64.60 ± 9.33 | 65.28 ± 10.71 | 0.285                              | 64.53 ± 12.57 | 0.940        |                                    |
| Gender (males), n (%)         | 331 (57.0) | 283 (67.1)   | 0.001                              | 98 (66.7) | 0.033        |                                    |
| Smoking, n (%)                | 133 (22.9) | 141 (33.4)   | 2.23 \times 10^{-4}               | 37 (25.2) | 0.560        |                                    |
| Hypertension, n (%)           | 251 (43.2) | 318 (75.4)   | 3.42 \times 10^{-24}              | 93 (63.3) | 1.34 \times 10^{-5} |                                    |
| Diabetes mellitus, n (%)      | 125 (21.5) | 158 (37.4)   | 3.15 \times 10^{-8}               | 37 (25.2) | 0.341        |                                    |
| Hypercholesterolemia, n (%)   | 195 (33.6) | 171 (40.5)   | 0.024                              | 49 (33.3) | 0.958        |                                    |

*Age values are means ± SD.

Table 2. Multivariate logistic regression analysis of CELSR1 polymorphisms related to ischaemic stroke and stroke sub-types.

| Characteristic | Cases | Controls | Crude OR (95% CI) | Crude \( p \) value | Adjusted OR* (95% CI) | Adjusted \( p \) value* |
|----------------|-------|----------|-------------------|---------------------|-----------------------|------------------------|
| Overall        |       |          |                   |                     |                       |                        |
| rs6007897 (Hardy–Weinberg \( p = 0.75 \)) |       |          |                   |                     |                       |                        |
| AA             | 535   | 565      | 0.007             |                     |                       |                        |
| AG             | 34    | 16       | 0.01              |                     |                       |                        |
| GG             | 0     | 0        | 0.007             |                     |                       |                        |
| rs4044210 (Hardy–Weinberg \( p = 0.75 \)) |       |          |                   |                     |                       |                        |
| AA             | 533   | 565      | 0.004             |                     |                       |                        |
| AG             | 36    | 16       | 0.01              |                     |                       |                        |
| GG             | 0     | 0        | 0.004             |                     |                       |                        |
| rs6007897 LAA  |       |          |                   |                     |                       |                        |
| AA             | 391   | 565      | 0.001             |                     |                       |                        |
| AG             | 31    | 16       | 0.001             |                     |                       |                        |
| GG             | 0     | 0        | 0.001             |                     |                       |                        |
| rs4044210 SAO  |       |          |                   |                     |                       |                        |
| AA             | 389   | 565      | < 0.001           |                     |                       |                        |
| AG             | 33    | 16       | 0.001             |                     |                       |                        |
| GG             | 0     | 0        | 0.001             |                     |                       |                        |
| *Multivariable logistic regression analysis was performed with adjustment for age, gender, smoking status and prevalence of hypertension, diabetes mellitus and hypercholesterolemia.
the adjusted ORs) genotypic tests revealed that neither rs6007897 nor rs4044210 of CELSR1 were related with hypertension (OR = 1.21, 95% CI = 0.66–2.21; OR = 1.27, 95% CI = 0.70–2.30), hypercholesterolemia (OR = 1.24, 95% CI = 0.69–2.24; OR = 1.25, 95% CI = 0.70–2.22) or type 2 diabetes mellitus (OR = 0.73, 95% CI = 0.37–1.43; OR = 0.76, 95% CI = 0.39–1.47).

Discussion

Two SNPs of CELSR1 rs6007897 and rs4044210 were previously identified as IS risk factors in a phased GWAS performed on Japanese individuals. Replication of GWAS association findings remains the gold standard for results validation. Our results showed that rs6007897 and rs4044210 were significantly associated with IS in a Chinese Han population and the variant G allele of rs6007897 and G allele of rs4044210 of CELSR1 were risk factors for this condition. The present study, along with the investigations on Japanese and Portuguese cohorts, provides further support for the importance of CELSR1 in IS (Gouveia et al., 2011; Yamada et al., 2009). In addition, we found that hypertension, hypercholesterolemia or diabetes mellitus are not linked with rs6007897 and rs4044210. The results suggest that these SNPs of CELSR1 may be independent risk factors of IS.

Studies show genetics for cardioembolic and non-cardioembolic stroke are different (Meschia, 2011). Yamada et al. (2009), who studied rs6007897 and rs4044210, excluded cardioembolic stroke in his study. Sample sizes for stroke with other determined or undetermined aetiology are small in our region. So we only included larger artery atherosclerosis (LAA) and small artery occlusion (SAO). Our results showed significant association of rs6007897 and rs4044210 of CELSR1 with IS sub-type LAA, but not with sub-type SAO. This may be due to the diversity of the mechanisms of SAO and the limitation of sample size of SAO in our study. SAO is usually attributed to lipohyalinotic small-vessel disease, cerebral amyloid angiopathy, microatheroma of perforators and atherothrombotic lesions at the parental artery. In addition, G allele frequency of these two SNPs is less than 3%, the negative results might be contributed by a smaller sample size in SAO (n = 147). Further study is needed to include larger sample sizes of SAO.

CELSR1 is a large 35-exon gene, mapping to chromosome 22q13.31 (Formstone et al., 2010) which encodes a cadherin with an atypical combination of seven-pass transmembrane domains, extracellular epidermal growth factor-like and laminin-G like domains crowned by nine protocadherin repeats. Celsr1 is a key component of the non-canonical Wnt/PCP pathway, which regulates cell migration and polarity in a variety of tissues (Crompton et al., 2007; Yates et al., 2010). The non-canonical Wnt/PCP pathway was recently found to play a critical role in endothelial cell proliferation, migration and angiogenesis (Ju et al., 2010). The zebrafish Wnt5 mutant (pipetail), which has impaired PCP signalling, displays vascular developmental defects (Cironne et al., 2008). In addition, Wnt5a is expressed in murine and human atherosclerotic lesions, indicating the involvement of the non-canonical Wnt/PCP pathway in atherosclerosis. Moreover, cell migration defects can contribute to the pathogenesis of many diseases including vascular diseases, such as atherosclerosis (Carmona-Fontaine et al., 2008). The rs4044210 is located in the hormone receptor domain of CELSR1, an extracellular domain containing four conserved cysteine residues that are likely to form disulphide bridges and which may function as a ligand-binding domain.

In conclusion, the present study has proven for the first time that CELSR1 is a susceptibility gene for IS in the Chinese Han population. Both rs6007897 and rs4044210 variants of CELSR1 were significantly associated with LAA subjects. Additional functional studies of the Celsr1 protein and the involvement of the non-canonical Wnt/PCP pathway in LAA will help to understand the relevance of the described variants to the susceptibility of IS, especially of LAA. This may further inform the diagnosis and therapy of IS and its sub-type, LAA.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary material available online

Supplementary Tables 1.