Association of genetic variants with coronary artery disease and ischemic stroke in a longitudinal population-based genetic epidemiological study

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Received February 2, 2015; Accepted February 10, 2015

DOI: 10.3892/br.2015.440

Abstract. Our previous studies identified nine genes and chromosomal region 3q28 as susceptibility loci for myocardial infarction, ischemic stroke or chronic kidney disease by genome-wide or candidate gene association studies. As coronary artery disease (CAD) and ischemic stroke may share genetic architecture, certain genetic variants may confer susceptibility to the two diseases. The present study examined the association of 13 polymorphisms at these 10 loci with the prevalence of CAD or ischemic stroke in community-dwelling individuals, with the aim of identifying genetic variants that confer susceptibility to the two conditions. Study subjects (170 with CAD, 117 with ischemic stroke and 5,718 controls) were recruited to the Inabe Health and Longevity Study, a longitudinal genetic epidemiological study of atherosclerotic, cardiovascular and metabolic diseases. The subjects were recruited from individuals who visited for an annual health checkup and they were followed up each year (mean follow-up period, 5 years). Longitudinal analysis with a generalized estimating equation, and with adjustment for age, gender, body mass index, smoking status, the prevalence of hypertension, diabetes mellitus and dyslipidemia and the serum concentration of creatinine, revealed that rs2074380 (G→A) of the α-kinase 1 (ALPK1) gene and rs8089 (T→G) of the thrombospondin 2 (THBS2) gene were significantly (P<2x10-4) associated with the prevalence of CAD, with the AA genotype of rs2074380 and GG genotypes of rs2074381 and rs8089 being protective against this condition. Similar analysis revealed that rs9846911 (A→G) at chromosome 3q28, rs2074381 of ALPK1, rs8089 of THBS2 and rs6046 (G→A) of the coagulation factor VII gene were significantly (P<2x10-4) associated with the prevalence of ischemic stroke, with the GG genotypes of rs9846911, rs2074381 and rs8089 and the AA genotype of rs6046 being protective against this condition. ALPK1 and THBS2 may thus be susceptibility loci for CAD and ischemic stroke.

Introduction

Coronary artery disease (CAD) is an important clinical problem due to its large contribution to mortality. In the United States, the total number of individuals affected by CAD or myocardial infarction (MI) was 15.5 and 7.6 million, respectively, in 2012. The annual incidence of new or recurrent MI and fatal CAD was 935,000, with an annual mortality of 375,295 from these conditions, in 2011 (1). Despite recent advances in therapy, such as drug-eluting stents (2) for acute coronary syndrome, CAD remains the leading cause of fatality in the United States (1). Disease prevention is an important strategy for reducing the overall burden of CAD and the identification of biomarkers for disease risk is key for risk prediction and potential intervention to reduce the chance of future coronary events.

Stroke is a complex multifactorial disorder that is believed to result from an interaction between the genetic background of an individual and various environmental factors. It is a common and serious condition, with ~795,000 individuals experiencing a new or recurrent stroke and 128,932 fatalities from stroke-related causes in 2011 in the United States. The prevalence of stroke in the United States was 6.6 million in 2012; 87% were ischemic stroke, 10% were intracerebral hemorrhage and 3% were subarachnoid hemorrhage (1). Regardless of recent advances in acute stroke therapy, stroke remains the leading cause of severe disability (3) and the fourth leading cause of fatality, after heart disease, cancer and chronic lower respiratory disease in the United States (1). The identification of biomarkers of stroke risk is important for risk prediction and intervention to avert future cerebrovascular events.
CAD and ischemic stroke (particularly atherothrombotic cerebral infarction) are atherosclerotic diseases that share various aspects of their underlying pathogenesis, as well as several risk factors, including hypertension, diabetes mellitus, dyslipidemia, chronic kidney disease and smoking (1). Twin and family studies have shown that CAD (4,5) and ischemic stroke (6,7) are highly heritable, with evidence of a shared heritability for the two conditions (8). Previous genome-wide association studies (GWASs) and meta-analyses of such studies have indicated various genes and loci in the predisposition to CAD or MI (9-15) or to ischemic stroke (16-21) in Caucasian populations. Furthermore, certain genetic variants originally shown to influence the risk of CAD were also subsequently found to be associated with ischemic stroke (22-24), suggestive of a shared genetic architecture for these conditions.

Although CAD and ischemic stroke may share genetic factors in Caucasian populations, the genes that confer susceptibility to the two conditions in Japanese individuals have not been identified. Our previous studies identified nine genes and chromosomal region 3q28 as susceptibility loci for MI, ischemic stroke or chronic kidney disease in Japanese individuals by genome-wide (25-27) or candidate gene (28-30) association studies. The aim of the present study was to examine the association of 13 polymorphisms at these 10 loci with the prevalence of CAD or ischemic stroke, as well as to identify polymorphisms that confer susceptibility to these conditions in community-dwelling Japanese individuals.

Materials and methods

Study population. Study subjects (170 with CAD, 117 with ischemic stroke and 5,718 controls) comprised community-dwelling individuals who were recruited to a population-based cohort study in Inabe (Inabe Health and Longevity Study), Mie, Japan. The Inabe Health and Longevity Study is a longitudinal genetic epidemiological study of atherosclerotic, cardiovascular and metabolic diseases (31-37). Detailed methods for recruitment of subjects and for collection and storage of medical examination data and genomic DNA samples were described previously (31).

In the CAD analysis, 170 subjects with CAD, including 88 with MI and 5,718 controls were examined. All the subjects with CAD underwent coronary angiography. The diagnosis of CAD was based on the detection of stenosis of ≥50% in any major coronary artery by coronary angiography. The diagnosis of MI was based on typical electrocardiographic changes and on increases in the serum activity of creatine kinase (MB isozyme) and in the serum concentration of troponin T. The diagnosis was confirmed by identification of the responsible stenosis in any of the major coronary arteries or in the left main trunk by coronary angiography. For the stroke analysis, 117 subjects with ischemic stroke and 5,718 controls were examined. The diagnosis of ischemic stroke was based on the occurrence of a new and abrupt focal neurological deficit, with neurological symptoms and signs persisting for ≥24 h; it was confirmed by positive findings in computed tomography or magnetic resonance imaging (or both) of the head. Individuals with cardiogenic embolic infarction, lacunar infarction alone, transient ischemic attack, hemorrhagic stroke, cerebrovascular malformations, moyamoya disease, cerebral venous sinus thrombosis, brain tumors or traumatic cerebrovascular diseases were excluded from the study. The control individuals had no history of CAD, aortic aneurysm or peripheral arterial occlusive disease; of ischemic or hemorrhagic stroke, or other cerebrovascular diseases; or of other atherosclerotic, thrombotic, embolic or hemorrhagic disorders.

The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine and Inabe General Hospital (Mie, Japan). Written informed consent was obtained from all the subjects.

Selection and genotyping of polymorphisms. The 13 single-nucleotide polymorphisms (SNPs) examined in the present study were selected from our previous genome-wide (25-27) or candidate gene (28-30) association studies as previously described (31). Wild-type (ancestral) and variant alleles of the SNPs were determined from the SNP database (dbSNP; National Center for Biotechnology Information, Bethesda, MD, USA).

Venous blood (5 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), peripheral blood leukocytes were isolated and genomic DNA was extracted from these cells with a DNA extraction kit (SMITEST EX-R&D; Medical and Biological Laboratories Co., Ltd, Nagoya, Japan). Genotypes of 13 SNPs were determined at G&G Science Co., Ltd. (Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes and other conditions for genotyping of SNPs examined in the present study were previously described (31). Detailed genotyping methodology was also described previously (38).

Statistical analysis. Quantitative data were compared between subjects with CAD or ischemic stroke and controls with the unpaired Student’s t-test. Categorical data were compared using the χ² test. The associations of 13 SNPs to the prevalence of CAD or ischemic stroke were examined in a 5-year longitudinal cohort study. Longitudinal changes in the prevalence of CAD or ischemic stroke were compared between two groups (dominant or recessive genetic model) by a generalized estimating equation (39) with adjustment for age, gender, body mass index, smoking status, the prevalence of hypertension, diabetes mellitus and dyslipidemia, and the serum concentration of creatinine. P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed with R software version 3-0-2 (The R Project for Statistical Computing) and JMP Genomics version 6.0 (SAS Institute Inc., Cary, NC, USA).

Results

Subject characteristics. Characteristics of subjects with CAD or ischemic stroke and controls in the cross-sectional analysis in March 2014 are shown in Table I. Age and the frequency of males were significantly greater in subjects with CAD or ischemic stroke compared to the controls.
The association of the 13 SNPs with the prevalence of CAD or ischemic stroke was analyzed with a generalized estimating equation with adjustment for age, gender, body mass index, smoking status, the prevalence of hypertension, diabetes mellitus and dyslipidemia, and the serum concentration of creatinine. In the CAD analysis, rs2074380 (G→A) and rs2074381 (A→G) of the α-kinase 1 gene (ALPK1), as well as rs8089 (T→G) of the thrombospondin 2 gene (THBS2) were significantly (P<0.05, recessive model) associated with the prevalence of CAD (Table II). Genotype distributions for rs2074380, rs2074381 and rs8089 in subjects with CAD and controls in a 5-year longitudinal study are shown in Table III. The AA genotype of rs2074380 and the GG genotypes of rs2074381 and rs8089 were protective against CAD. In the stroke analysis, rs2116519 (C→T) of the family with sequence similarity 78-member B gene (FAM78B), rs9846911 (A→G) at chromosome 3q28, rs2074381 (A→G) of ALPK1, rs8089 (T→G) of THBS2 and rs6046 (G→A) of the coagulation factor VII (F7) gene were significantly (P<0.05, recessive model) associated with the prevalence of ischemic stroke (Table IV). Genotype distributions for these SNPs in subjects with ischemic stroke and controls in a 5-year longitudinal study are shown in Table V.
Atherosclerosis is the main cause of CAD and ischemic stroke. The principal and treatable risk factors include hypertension, diabetes mellitus, dyslipidemia, chronic kidney disease and smoking (1). In addition to these conventional risk factors, genetic variants are important in the pathogenesis of CAD (4,5) and ischemic stroke (6,7). Prediction of the risk for these conditions beyond the usual clinical risk factors is required as genetic variants would be useful for deciding how aggressively to target the risk factors that are currently responsive to treatment. The present study showed that rs2074381 (A–G)
of ALPK1 and rs8089 (T→G) of THBS2 were significantly associated with the prevalence of CAD and ischemic stroke in community-dwelling Japanese individuals.

ALPK1 functions in apical transport by phosphorylating myosin la in epithelial cells and is indicated in the regulation of intracellular trafficking processes by phosphorylation (40). ALPK1 may act synergistically with monosodium urate monohydrate crystals to promote the production of proinflammatory cytokines through the activation of nuclear factor-κB and mitogen-activated protein kinase (extracellular signal-regulated kinase 1/2 and p38) signaling in cultured human embryonic kidney 293 (HEK293) cells, suggesting that ALPK1 may contribute to the inflammatory process associated with the development of gout (41). Our previous GWAS for chronic kidney disease showed that the overexpression of ALPK1 resulted in upregulation of the expression of cystatin C in cultured HEK293T cells (27). Cystatin C is an inhibitor of cysteine proteases and is recognized as a sensitive marker of renal dysfunction (42). Cystatin C is also associated with inflammation regardless of renal function. The serum concentration of cystatin C was thus associated with those of C-reactive protein and fibrinogen in 990 subjects with coronary heart disease in the Heart and Soul Study (43), as well as in subjects with renal dysfunction in the Cardiovascular Health Study (44). Furthermore, the serum concentration of cystatin C was associated with the prevalence and severity of CAD (45-47), the risk of secondary cardiovascular events (48,49) and cardiovascular mortality (50). Cystatin C was also associated with ischemic and hemorrhagic stroke (51) and subclinical cerebral infarction (52). These observations suggest that the association of ALPK1 with CAD and ischemic stroke may be attributable, at least in part, to the effects of cystatin C on the development of atherosclerosis. rs2074381 of ALPK1 was significantly associated with CAD and ischemic stroke, with the GG genotype being protective against these conditions. These previous observations (27,41,43-52) and our present results suggest that ALPK1 may contribute to the development of CAD and ischemic stroke through the acceleration of vascular inflammation, although the molecular mechanisms underlying the role of rs2074381 of ALPK1 in the pathogenesis of CAD and ischemic stroke remain unknown.

Thrombospondins are calcium-binding glycoproteins of the extracellular matrix. They support cell attachment through interactions with multiple cell-adhesion receptors, bind to other extracellular matrix components and regulate cell shape, adhesion and migration, which in turn influence more complex biological processes, such as angiogenesis and wound healing (53). THBS2 is a multifunctional protein that contributes to the control of smooth muscle cell growth in an autocrine manner (54). THBS2 has a role in extracellular matrix organization, as suggested by the observation that disruption of THBS2 in mice results in a complex phenotype characterized by fibroblast, connective tissue and blood vessel abnormalities that are similar to those associated with Ehlers-Danlos syndrome type IV (55). Furthermore, a two-fold increase in matrix metalloproteinase 2 (MMP2) activity was found to contribute to the adhesive defect that manifested in THBS2-null fibroblasts (56). Loss of THBS2 may result in an increase in MMP2 levels in the pericellular environment, as THBS2 is capable of binding the pro- and mature forms of this protease (57). A T→G variant in the 3′ untranslated region of THBS2 was previously shown to have a protective effect against familial premature MI in the United States, with individuals homozygous for the variant having an odds ratio of 0.31 (58). The G allele of this polymorphism was also associated with a reduced risk of premature MI among individuals in the Netherlands, possibly as a result of an increase in MMP2 activity (59). In the present study, rs8089 of THBS2 was significantly associated with CAD and ischemic stroke, with the GG genotype being protective against these conditions. Previous studies (53-59) and our present observations suggest that the interaction between THBS2 and MMP2 may play an important role in the predisposition to CAD and ischemic stroke, although the molecular mechanisms underlying the role of rs8089 of THBS2 in the pathogenesis of these conditions remain unclear.

rs2116519 of FAM78B, rs9846911 at 3q28 and rs6046 of F7 were also associated with the prevalence of ischemic stroke. FAM78B is located at 1q24.1, which was previously suggested to harbor susceptibility loci for hypertension (60) and type 2 diabetes mellitus (61), which are important risk factors for ischemic stroke, although the function of the gene remains unclear. The rs9846911 SNP at 3q28 was previously identified as a susceptibility locus for chronic kidney disease, which is also a risk factor for ischemic stroke in Japanese individuals (27). The functional role of this chromosomal region remains to be elucidated. F7 encodes coagulation factor VII, which is a vitamin K-dependent factor essential for hemostasis. The rs6046 (G→A, Arg353Gln) SNP of F7 was previously associated with the levels of factor VII antigen and activity (62), as well as with prothrombin time (63). Our previous study showed that rs6046 of F7 was associated with MI in individuals with chronic kidney disease, with the minor A allele being protective against this condition, in a hospital-based study (29). Our present results are thus consistent with this previous observation (29).

There are limitations to the present study: i) As the study subjects comprised only Japanese individuals, further studies will be required in other ethnic groups. ii) rs2074381 of ALPK1 or rs8089 of THBS2 are possibly in linkage disequilibrium with other polymorphisms in the same gene or in nearby genes that are actually responsible for the development of CAD or ischemic stroke. iii) The functional relevance of rs2074381 or rs8089 to the pathogenesis of CAD or ischemic stroke remains unclear.

In conclusion, the present results suggest that ALPK1 and THBS2 may be susceptibility loci for CAD and ischemic stroke in Japanese individuals. Determination of the genotype for rs2074381 and rs8089 may prove informative for assessment of the genetic risk for CAD and ischemic stroke in such individuals.

Acknowledgements

The present study was supported by the Core Research for Evolutional Science and Technology of the Japan Science and Technology Agency (to Y.Y. and I.T.), as well as by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant no. 24590746 to Y.Y.).
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