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Report

Association between the Gene Encoding 5-Lipoxygenase–Activating Protein and Stroke Replicated in a Scottish Population

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Cardiovascular diseases, including myocardial infarction (MI) and stroke, most often occur on the background of atherosclerosis, a condition attributed to the interactions between multiple genetic and environmental risk factors. We recently reported a linkage and association study of MI and stroke that yielded a genetic variant, HapA, in the gene encoding 5-lipoxygenase–activating protein (*ALOX5AP*), that associates with both diseases in Iceland. We also described another *ALOX5AP* variant, HapB, that associates with MI in England. To further assess the contribution of the *ALOX5AP* variants to cardiovascular diseases in a population outside Iceland, we genotyped seven single-nucleotide polymorphisms that define both HapA and HapB from 450 patients with ischemic stroke and 710 controls from Aberdeenshire, Scotland. The Icelandic at-risk haplotype, HapA, had significantly greater frequency in Scottish patients than in controls. The carrier frequency in patients and controls was 33.4% and 26.4%, respectively, which resulted in a relative risk of 1.36, under the assumption of a multiplicative model ($P = .007$). We did not detect association between HapB and ischemic stroke in the Scottish cohort. However, we observed that HapB was overrepresented in male patients. This replication of haplotype association with stroke in a population outside Iceland further supports a role for *ALOX5AP* in cardiovascular diseases.

Cardiovascular diseases (CVDs), such as coronary heart disease and stroke, are major causes of death and disability in western societies (Aboderin et al. 2002). As a result of the increasing age of the population, the prevalence of CVD is rising worldwide (American Heart Association 2002). CVDs are largely attributed to atherosclerosis, which has various environmental and genetic risk factors. It is a commonly held view that chronic inflammation initiates and promotes the development of atherosclerotic lesions (Lusis 2000; Libby 2002). Large epidemiologic studies have demonstrated correlations between increased production of markers of systemic inflammation and future cardiovascular events, including myocardial infarction (MI) (Ridker et al. 1997, 1998; Danesh et al. 2000) and stroke (Di Napoli et al. 2001), which supports a central role for inflammation in CVD.

We recently published the association of a variant in the gene encoding 5-lipoxygenase–activating protein (*ALOX5AP* [MIM 603700]) with both MI and stroke in an Icelandic population (Helgadottir et al. 2004). *ALOX5AP,* which encodes an important component of the leukotriene pathway, was identified through a genomewide linkage scan conducted on 296 families with MI and subsequent analysis that determined association with markers within the mapped region on chromosome 13q12-13. A haplotype spanning *ALOX5AP,* HapA, defined by four SNPs, was shown to be associated with MI (relative risk = 1.8; $P = .0000023$) and, subsequently, the same variant was found to confer risk of stroke in Iceland (relative risk [RR] = 1.7; $P = .000095$) (Helgadottir et al. 2004). Another SNP-based haplotype within *ALOX5AP,* HapB, showed significant association with MI in British cohorts from Leicester and Sheffield (RR = 2.0; $P = .00037$) (Helgadottir et al. 2004). We further demonstrated that leukotriene B4 (LTB4) synthesis by neutrophils from patients with a history of MI

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is greater than the synthesis by those from controls without MI (Helgadottir et al. 2004).

In the present study, we attempted to replicate the association of *ALOX5AP* with stroke in a population outside Iceland. The SNPs defining HapA (*SG13S25, SG13S114, SG13S89,* and *SG13S32*) and HapB (*SG13S377, SG13S114, SG13S41,* and *SG13S35*) were genotyped for 450 Scottish patients who had experienced a stroke and for 710 controls. The patient and control cohorts have been described elsewhere (MacLeod et al. 1999; Meiklejohn et al. 2001; Duthie et al. 2002; Whalley et al. 2004). In brief, 450 patients from northeastern Scotland with CT confirmation of ischemic stroke (including 26 patients with transient ischemic attack [TIA]) were recruited between 1997 and 1999, within 1 wk of admission to the Acute Stroke Unit at Aberdeen Royal Infirmary. Patients were further subclassified in accordance with the TOAST (Trial of Org 10172 in Acute Stroke Treatment) research criteria (Adams et al. 1993). Of the patients, 155 (34.4%) had large-vessel stroke, 96 (21.3%) had cardiogenic stroke, and 109 (24.2%) had small-vessel stroke; for 5 (1.1%) of the patients, stroke with other determined etiology was diagnosed, 7 (1.6%) had more than one etiology, and 78 (17.3%) had unknown cause of stroke despite extensive evaluation. A total of 710 control individuals with no history of stroke or TIA were recruited during follow-up of the 1921 ($n = 227$) and 1936 ($n = 371$) Aberdeen Birth Cohort Studies originally recruited in 1932 and 1947, respectively, as part of the Scottish mental surveys (Deary et al. 2004). A further 112 controls were recruited from local primary-care practices (Meiklejohn et al. 2001). Basic clinical characteristics of patients and control individuals are shown in table 1. Approval for the study was granted by the local research ethics committee, and all study participants gave written informed consent.

The haplotype analysis was performed using the program NEMO (Gretarsdottir et al. 2003). NEMO handles missing genotypes and uncertainty with phase through a likelihood procedure, by use of the expectation-maximization algorithm as a computational tool to estimate haplotype frequencies. Since we were testing only two haplotypes, which had been shown elsewhere to confer risk of MI and stroke in an Icelandic cohort and MI in an English cohort, the reported *P* values are one sided. For the at-risk haplotypes, we calculated RR and population-attributable risk (PAR) under the assumption of a multiplicative model (Falk and Rubinstein 1987; Terwilliger and Ott 1992) in which the risk of the two alleles of haplotypes a person carries multiplies.

The results of the haplotype-association analysis for HapA and HapB are shown in table 2. The haplotype frequencies of HapA in the Scottish populations (patient and control) were higher than in the corresponding Icelandic populations (table 2). As demonstrated in the Icelandic population, the estimated frequency of HapA was significantly greater in Scottish patients who have suffered a stroke than in Scottish controls. The carrier frequency of HapA in Scottish patients and controls was 33.4% and 26.4%, respectively, which resulted in an RR of 1.36 ($P = .007$) and a corresponding PAR of 9.6%. We had previously observed in the Icelandic population a higher frequency of HapA in male than in female patients with either stroke or MI (Helgadottir et al. 2004). This sex difference in the frequency of HapA was not observed in the Scottish population (table 2).

We then tested the association of HapB with stroke in the Scottish cohort. HapB has been shown elsewhere to confer risk of MI in an English cohort (Helgadottir et al. 2004). A slight excess of HapB was observed in the patient group (6.8%) compared with controls (5.8%), but it was not significant (table 2). However, sex-specific analysis showed that the frequency of HapB was higher in males with ischemic stroke (9.2%) than in controls, resulting in an RR of 1.65 ($P = .016$). The frequency of HapB in females with ischemic stroke was 3.5%, which was lower but not significantly different from that of controls. The frequencies of HapB in males and females with ischemic stroke differed significantly ($P = .0021$). Interestingly, as shown in table 2, similar trends were observed in our Icelandic cohort; the frequency of HapB was greater in males with ischemic stroke (8.6%) than in females with ischemic stroke (5.8%), although this was not significant ($P = .055$).

To summarize our results, we demonstrate in the present study that HapA, the risk haplotype of *ALOX5AP,* reported elsewhere to confer risk of MI and stroke in an Icelandic cohort, associates with ischemic stroke in a Scottish cohort. HapB, which confers risk of MI in an English cohort, was not associated with ischemic stroke in the Scottish cohort. However, we observed that HapB was overrepresented in male patients.

Historical and archaeological data have suggested a Gaelic ancestry for both Icelanders and Scots. This is

Table 1

Clinical Characteristics of Scottish Patients and Control Individuals

Characteristics	Patients ($n = 450$)	Controls ($n = 710$)
Female:male	42:58	49:51
Age (years)	66.8 ± .6	67.2 ± .4
Hypertension (%)	55.5	23.9
Diabetes (%)	12.6	2.1
Total cholesterol (mmol/liter)	5.65 ± .06	5.64 ± .05

NOTE.—Patients and control individuals were classified as having hypertension and/or diabetes on the basis of previous history or receipt of antihypertensive or antidiabetic therapy. Values with plus-minus symbol (±) are mean ± SE.

Table 2

Analysis of Association of HapA and HapB with Ischemic Stroke

	HapA			HapB		
Location and Study Population (*n*)	Frequency	RR	*P*	Frequency	RR	*P*
Scotland:						
Controls (710)	.142			.058		
Patients with ischemic stroke (450[a]):	.184	1.36	.007	.068	1.20	NS
Males (253)	.183	1.35	.023	.092	1.65	.016
Females (181)	.179	1.34	.044	.035	.58	NS
Iceland:						
Controls (624)	.095			.067		
Patients with ischemic stroke (632):	.147	1.63	.00013	.073	1.09	NS
Males (335)	.155	1.75	.0002	.086	1.31	NS
Females (297)	.138	1.51	.0079	.058	.86	NS

Note.—Shown are HapA and HapB of *ALOX5AP* and the corresponding number of individuals genotyped, the haplotype frequency in the patient and control cohorts, the RR, and the one-sided *P* values. HapA is defined by the SNPs *SG13S25, SG13S114, SG13S89,* and *SG13S32,* with alleles G, T, G, and A, respectively, and HapB is defined by the SNPs *SG13S377, SG13S114, SG13S41,* and *SG13S35,* with alleles A, A, A, and G, respectively. For SNP genotyping, we used TaqMan assays (Applied Biosystems) or the fluorescent-polarization template-directed dye-terminator incorporation (the SNP-FP-TDI assay), as described elsewhere (Chen et al. 1999). SNP information can be found in the dbSNP database. The DNA used for the SNP genotyping was the product of whole-genome amplification, by use of the GenomiPhi Amplification kit (Amersham), of DNA isolated from the peripheral blood of the Scottish controls and patients with stroke. Data on the Icelandic cohort have been reported elsewhere (Helgadottir et al. 2004). NS = not significant.

[a] Sex unknown for 16 patients.

further supported by recent studies of mtDNA and Y-chromosome diallelic and microsatellite variation in Icelanders, Scandinavians, and Gaels from Ireland and Scotland (Helgason et al. 2000, 2001). Given this common ancestry, it is possible that the two populations share a disease-causing variant and that this variant may reside on the same common haplotype background (HapA). Such a scenario would be consistent with our results; although the estimated RR for HapA in the Scottish cohort is somewhat lower than in the Icelandic cohort, this difference is not statistically significant. Indeed, a similar observation has been made in previous studies of schizophrenia in Iceland and Scotland (Stefansson et al. 2003), in which the same extended haplotype was found to confer risk of schizophrenia in both populations, with comparable frequencies in patient and control groups in the two countries.

The gene *ALOX5AP* encodes the membrane-associated 5-lipoxygenase–activating protein (FLAP), an important mediator of the activity of cellular 5-lipoxygenase (5-LO), which is a key enzyme in the biosynthesis of leukotrienes (Dixon et al. 1990; Miller et al. 1990). Leukotrienes are proinflammatory mediators produced predominantly in inflammatory cells such as polymorphonuclear leukocytes, macrophages, and mast cells. Over the last decade, a number of studies have supported an important role for inflammation in atherosclerosis—from atheroma initiation to promotion of plaque rupture, thereby triggering thrombosis, the main atherosclerotic complication that causes MI and stroke (Libby 2002). The 5-LO pathway could be an important contributor to the pathophysiology of atherosclerosis through the formation of the proinflammatory LTB4 and/or through an increase in vascular permeability caused by cysteinyl leukotrienes. Indeed, we have shown increased production of LTB4 in neutrophils from patients with history of MI, compared with controls without history of MI (Helgadottir et al. 2004). This is further supported by recent human-expression studies (Spanbroek et al. 2003) that show an increased expression of members of the 5-LO pathway, including 5-LO and FLAP, in atherosclerotic lesions at various stages of their development. Moreover, a promoter variant of 5-LO (*ALOX5* [MIM 152390]) has been shown to be associated with increased carotid artery intima-media thickness and with heightened inflammatory biomarkers (Dwyer et al. 2004). In addition, an atherosclerotic mouse model with a heterozygous deficiency of 5-LO shows resistance to atherosclerosis (Mehrabian et al. 2002), and an LTB4 receptor antagonist blocks the development of atherosclerosis in apoE- and LDLR-deficient mice (Aiello et al. 2002; Mehrabian et al. 2002). Together, these studies suggest that chronic upregulation of the leukotriene pathway may be harmful to the vasculature, in terms of atherosclerosis progression and plaque instability.

The precise mechanism by which the *ALOX5AP* variants confer risk of MI and stroke is still unclear. As reported elsewhere, we have not observed SNPs in the coding sequence that led to amino acid substitution (Helgadottir et al. 2004). Therefore, one can speculate that

unidentified variation in regulatory regions of the gene—that affects transcription, splicing, message stability, message transport, or translation efficiency—may underlie the risk conferred by *ALOX5AP*.

The results of the present study show that HapA associates with ischemic stroke in a Scottish population, thereby providing replication of work that showed that the same haplotype confers increased risk of stroke in an Icelandic population. This replication constitutes additional evidence for the role of *ALOX5AP* in the pathogenesis of stroke. Identification of genetic risk factors for the common forms of stroke may facilitate identification of individuals at increased risk and may lead to novel strategies for the prevention and treatment of stroke.

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Electronic-Database Information

The URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *ALOX5AP* and *ALOX5*)

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