

ALOX5AP Gene and the *PDE4D* Gene in a Central European Population of Stroke Patients

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Background and Purpose—Recent evidence has implicated the genes for 5-lipoxygenase activating protein (*ALOX5AP*) and phosphodiesterase 4D (*PDE4D*) as susceptibility genes for stroke in the Icelandic population. The aim of the present study was to explore the role of these genes in a central European population of stroke patients.

Methods—A total of 639 consecutive stroke patients and 736 unrelated population-based controls that had been matched for age and sex were examined using a case-control design. Twenty-two single-nucleotide polymorphisms (SNPs) covering *ALOX5AP* were genotyped. For *PDE4D*, microsatellite AC008818-1 and 12 SNPs, which tag all common haplotypes in previously identified linkage disequilibrium (LD) blocks, were analyzed.

Results—A nominally significant association with stroke was observed with several SNPs from *ALOX5AP*, including SNP SG13S114, which had been part of the Icelandic at-risk haplotype. Associations were stronger in males than in females, with SG13S114 (odds ratio, 1.24; 95% CI, 1.04 to 1.55; $P=0.017$) and SG13S100 (odds ratio, 1.26; 95% CI 1.03 to 1.54; $P=0.024$) showing the strongest associations. No significant associations were detected with single markers and haplotypes in *PDE4D*. The frequencies of single-marker alleles and haplotypes differed largely from those in the Icelandic population.

Conclusions—The present study suggests that sequence variants in the *ALOX5AP* gene are significantly associated with stroke, particularly in males. Variants in the *PDE4D* gene are not a major risk factor for stroke in individuals from central Europe. Population differences in allele and haplotype frequencies as well as LD structure may contribute to the observed differences between populations. (*Stroke*. 2005;36:731-736.)

Key Words: genetics ■ stroke

Recently, the 5-lipoxygenase activating protein gene (*ALOX5AP*) on chromosome 13q and the phosphodiesterase 4D gene (*PDE4D*) on chromosome 5q have been reported to confer risk of stroke independently of conventional risk factors.¹⁻³ The candidacy of these genes was identified through genome-wide scans and subsequent case-control association studies in individuals from Iceland. Both genes have been implicated in atherosclerosis.

ALOX5AP encodes 5-lipoxygenase-activating protein, which is required for the synthesis of leukotrienes.⁴ Leukotrienes are secreted by various types of inflammatory cells that cluster at the injured sites in blood vessels and are implicated in the progression of atherosclerosis.⁵⁻⁷ In the study from DeCode, a single *ALOX5AP* haplotype (termed HapA) was found to double the risk of myocardial infarction (MI) and stroke in patients from Iceland. Another haplotype (termed HapB) was associated with MI in individuals from the United Kingdom, but patients with stroke were not investigated.³

PDE4D encodes phosphodiesterase 4D, which is a member of a large super-family of cyclic nucleotide phosphodiesterases. Phosphodiesterase 4D degrades second messenger cAMP, which is a key signal transduction molecule in different cell types, including inflammatory, vascular endothelial, and smooth muscle cells.⁸⁻¹⁰ Low levels of cAMP enhance cell migration and proliferation,¹¹ processes that are proatherogenic. Gretarsdottir et al² found that specific single markers and haplotypes at *PDE4D* are associated with carotid and cardiogenic stroke but not with other stroke subtypes.

Although of great potential importance, these findings need to be replicated in other populations. Therefore, we set up to evaluate the candidacy of *ALOX5AP* and *PDE4D* as susceptibility genes for stroke in a large population of stroke patients and matched controls from central Europe using single-marker and haplotype association tests and a case-control design.

Received October 22, 2004; final revision received November 29, 2004; accepted December 13, 2004.

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Stroke is available at <http://www.strokeaha.org>

DOI: 10.1161/01.STR.0000157587.59821.87

Subjects and Methods

Study Sample

The study population consisted of 639 patients (mean age 65 ± 18.2 years; 403 men) consecutively recruited from a single stroke unit (Department of Neurology, Klinikum Großhadern, University of Munich, Germany) that serves as a major referral center in the area covering a population of ≈ 4 million people. Thus, for practical purposes, these patients may be considered unrelated. All patients underwent computed tomography of the head for initial imaging and an ECG immediately after hospital admission. Duplex sonography of the carotid arteries was performed in all patients. A total of 492 (77%) patients received an MRI including diffusion-weighted images, which was usually performed within 3 days after admission. Magnetic resonance angiography was obtained in 251 (39%) cases. Transcranial Doppler sonography and transthoracic echocardiography were done in $>90\%$ and 60% of the patients, respectively. Holter monitoring and transesophageal echocardiography were done if appropriate.

Ischemic stroke was diagnosed in 601 patients and was further classified into stroke subtypes using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.¹² A total of 186 patients had large vessel disease (or "carotid stroke," as referred to by Gretarsdottir et al²), 143 had cardioembolic stroke (or "cardiogenic stroke"), and 86 had lacunar stroke. In 45 cases, stroke with other determined etiology was diagnosed, and in 141, the etiology remained unclear despite diagnostic efforts or competing etiologies were found. The remaining patients ($n=38$) had a hemorrhagic stroke.

The control group consisted of 736 unrelated individuals (mean age 62 ± 11.7 years; 447 men), who were selected from the KORAS2000 study, a community-based epidemiological project near Munich. Controls were sampled to match stroke patients for age and gender. Informed consent was obtained from all individuals (or relatives or legal guardians). Genomic DNA was extracted from EDTA blood using standard protocols. The study was approved by the local ethics committee.

Marker Selection and Genotyping

The nomenclature of markers used in the original studies was adopted and maintained throughout the current study.^{2,3} For *ALOX5AP*, we selected all 22 single-nucleotide polymorphisms (SNPs) that had been used for haplotype analysis in the original study by the DeCode group.³ They cover the entire *ALOX5AP* gene by a mean distance of 1.9 kb between SNPs. For *PDE4D*, we selected 2 SNPs (SNP45 and SNP41) and 1 microsatellite marker

(AC008818-1), which had shown the most significant association with stroke in the original study.² We further identified an optimal set of haplotype tagging SNPs (htSNPs) that distinguished 95% of all chromosomes within linkage disequilibrium (LD) blocks "B" and "C" using a nested algorithm implemented in the program SNPtagger.^{2,13} Eight htSNPs were from block B (SNP73, SNP69, SNP67, SNP57, SNP49, SNP48, SNP45, and SNP41) and 4 htSNPs from block C (SNP34, SNP30, SNP20, and SNP6).² Genotyping of SNPs was achieved by primer extension of multiplex polymerase chain reaction products with detection of allele-specific extension products by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF; Sequenom) mass spectroscopy. Microsatellite AC008818-1 was genotyped using fluorescently labeled oligonucleotide probes by ABI 3730 sequencer (Applied Biosystems). Amplification of AC008818-1 marker alleles was obtained by using the sense primer 5' TGCTTGGTGAAGGAATAGCC 3' and antisense primer 5' GAGCCTGGGTTCTCAGGAAT 3'. Marker sequences were obtained from the Genome database, and SNPs were selected from the initial publications.^{2,3} There were no deviations from Hardy-Weinberg equilibrium (tested by a χ^2 test), and all markers were genotyped with a high call rate (average 99%).

Statistical Methods

Allele and genotype frequencies were calculated for each locus and tested for Hardy-Weinberg equilibrium. Pairwise LD values were plotted using the software package Haploview (Barrett; Whitehead Institute, 2003). The program PHASE version 2.0 was used for haplotype frequency estimation, which uses a Bayesian method accounting for the evolutionary relationship of estimated haplotypes.¹⁴

Single-marker associations between phenotype and genotype were tested by logistic regression analyses (STATA statistical software package). The association of haplotypes was inferred by the haplotype trend regression method proposed by Zaykin et al.¹⁵ Individuals' haplotype probabilities estimated by the phase estimation procedure are regressed against the phenotype state. We tested 2-, 3-, 4- and all marker haplotypes from adjacent SNPs in a sliding-window fashion, as well as using all different 2-marker combinations.

Markers and haplotypes significantly associated in the original studies were evaluated by confirmatory testing using uncorrected *P* values. For markers or haplotypes not significantly associated in the original studies, experiment-wise significance levels were evaluated by a permutation procedure, which corrects for the number of tests performed in a given marker set.

The power of our case-control sample to detect a similarly sized effect as in the original studies was determined by using the reported

TABLE 1. Single-Marker Allelic Association in the *ALOX5AP* Gene With Markers Constituting HapA Reported Previously to be Associated With Stroke

Individuals (No. of cases/controls)	SNP	Public Name	Allele	Frequency (%)		OR	95% CI	<i>P</i> Value
				Cases	Controls			
All (639/736)	SG13S25		G	89.4	90.1	0.93	0.73–1.19	NS
	SG13S114	rs10507391	T	68.5	65.0	1.17	1.00–1.37	0.025
	SG13S89	rs4769874	G	94.7	96.0	0.75	0.53–1.07	NS
	SG13S32		A	46.9	49.7	1.15	0.99–1.34	NS
Males (408/447)	SG13S25		G	89.8	89.7	1.00	0.74–1.37	NS
	SG13S114	rs10507391	T	69.2	63.8	1.24	1.04–1.55	0.017
	SG13S89	rs4769874	G	94.9	95.4	0.91	0.59–1.41	NS
	SG13S32		A	46.5	47.9	1.25	1.03–1.5	0.022
Females (241/289)	SG13S25		G	88.8	90.1	0.82	0.54–1.23	NS
	SG13S114	rs10507391	T	69.2	67.0	0.94	0.74–1.23	NS
	SG13S89	rs4769874	G	94.3	96.9	0.52	0.28–0.97	0.040
	SG13S32		A	47.5	47.6	1.00	0.98–1.29	NS

OR indicates odds ratio.

TABLE 2. Association of HapA (ALOX5AP) to Stroke

	German Sample			Icelandic Sample ²		
	% Cases	% Controls	<i>P</i> value	% Cases	% Controls	<i>P</i> value
HapA*	14.5	15.2	NS	14.9	9.5	0.000095

*Constituted by SNPs SG13S25 (G allele), SG13S114 (T allele), SG13S89 (G allele), and SG13S32 (A allele).³

relative risks and risk allele frequencies^{2,3} at a 5% type I error rate under a multiplicative model.¹⁶

Results

ALOX5AP

We first performed single-marker association tests with the 4 SNPs (SG13S25, SG13S114, SG13S89, and SG13S32) constituting HapA (ie, the haplotype that was associated with stroke and MI in patients from Iceland).³ As shown in Table 1, the T allele of marker SG13S114 was more frequent in our stroke cases compared with controls ($P=0.025$). This effect was most significant in males and not significant in women. Subgroup analysis for stroke subtypes revealed no significant association with stroke subtypes (data not shown).

Next, we looked at the HapA haplotype. As shown in Table 2, there was no significant association with HapA in our continental population of stroke patients. Also, association tests with males or females separately and with different stroke subtypes revealed no significant associations. Contrasting with the Icelandic study, HapA occurred more often in controls than in cases. Also, the frequency in controls was considerably higher than in the Icelandic population (Table 2). We also looked at the HapB haplotype, which was associated with MI in individuals from the United Kingdom.³ However, no significant association was found with the stroke phenotype in our sample (data not shown).

Because we could not replicate associations with HapA, we expected that there might be other unidentified haplotypes in *ALOX5AP* that confer risk to stroke in our population. Therefore, we performed different haplotype combination tests constructed from 2, 4, and all SNPs. Several haplotypes were significantly associated with stroke (minimum P value=0.004; supplemental Table I, available online at <http://www.strokeaha.org>; data not shown). However, after correction for multiple testing, associations were no longer significant. Single-marker analysis with the remaining SNPs (not included in HapA) revealed nominally significant associations between several markers (SG13S100, SG13S34, SG13S86, and SG13S96) and overall stroke (Table II, available online at <http://www.strokeaha.org>). However, after correction for multiple testing, associations were no longer significant.

To check for possible differences in LD structure between our German and the Icelandic sample, we determined the LD structure and haplotype diversity of *ALOX5AP* in our population (Table I). Most SNPs were in strong LD, and the LD block structure was similar to that in the Icelandic population (Figure 1).³

PDE4D

We started by performing single-marker association tests with SNP45, SNP41, and AC008818-1, which had shown the most significant associations with the combined carotid and cardiogenic stroke subtypes in the Icelandic population.² No association was found with the overall population of stroke patients, the combined phenotype, or any of the stroke subtypes (Table 3 and data not shown). Again, the frequency of the original at-risk alleles was considerably higher in our controls than in the Icelandic control sample.²

Next, we constructed haplotypes using the same combination of markers (AC008818-1 and SNP45), which had shown

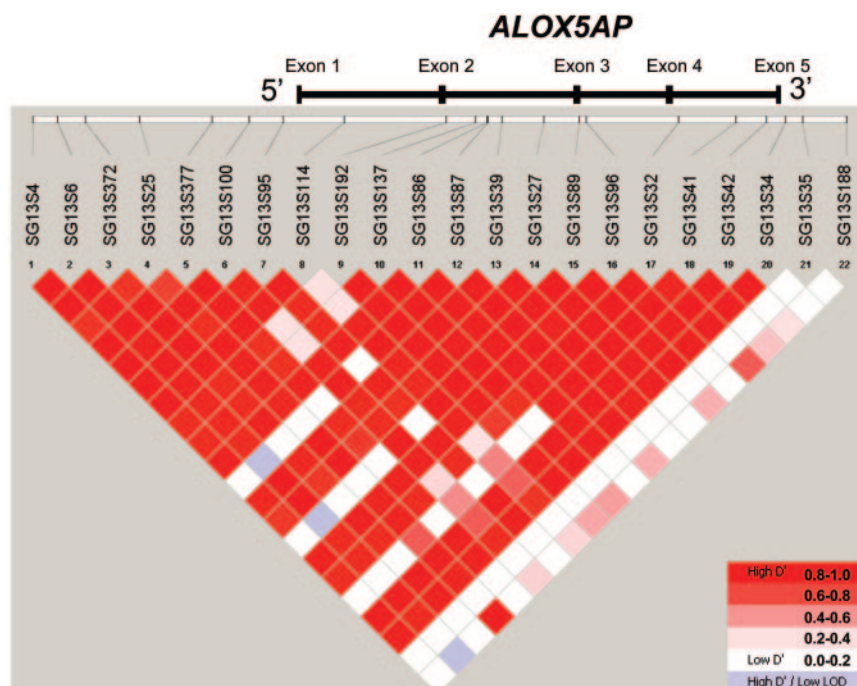


Figure 1. Pairwise LD between SNPs in the *ALOX5AP* gene region. A color-coded scale for D' (a measure of LD strength) is provided.

TABLE 3. Single-Marker Allelic Association in the *PDE4D* Gene With Markers Reported Previously to be Associated With Combined Carotid and Cardiogenic Stroke

Stroke phenotype (No. of cases/controls)	Marker	At-Risk Allele	Frequency (%)		OR	95% CI	P Value
			Cases	Controls			
All patients (639/736)	SNP45	G	86.1	86.3	0.96	0.78–1.18	NS
	SNP41	A	89.8	89.4	0.97	0.76–1.24	NS
	AC008818	O*	29.2	29.0	0.95	0.82–1.12	NS
Combined carotid and cardiogenic (n=329/736)	SNP45	G	87.5	86.3	1.11	0.85–1.45	NS
	SNP41	A	90.2	89.4	0.91	0.66–1.24	NS
	AC008818	O	26.0	29.0	1.02	0.84–1.25	NS

OR indicates odds ratio.

*At-risk allele in the Icelandic population.³

significant association with the combined carotid and cardiogenic stroke phenotypes in the Icelandic population. However, no associations were found (Table 4). In addition, we looked at all possible haplotypes constructed from 2, 3, 4, or all SNPs from blocks B and C using the full set of htSNPs. Again, no significant associations were found after correction for multiple testing (Table III, available online at <http://www.strokeaha.org>; data not shown). Single-marker analysis with the remaining markers (all but SNP45, SNP41, and AC008818-1) revealed nominally significant associations between SNP73 and cardiogenic stroke and between SNP57 and carotid stroke and the combined carotid and cardiogenic stroke subtype (Table IV, available online at <http://www.strokeaha.org>; data not shown). However, after correction for multiple comparisons, these associations were not significant.

To check for possible differences in LD structure between our German and the Icelandic sample, we determined the LD structure and haplotype diversity of *PDE4D* in our population (Table III). The LD between SNPs was less pronounced than in the Icelandic population, and the LD block structure slightly differed from that reported by DeCode (Figure 2).²

Discussion

In this study, we applied a 2-step replication approach. We first examined the most significant single markers and haplotypes that were associated with stroke in the Icelandic

population. Analysis was then extended to additional markers and haplotypes.

We found no association with the Icelandic at-risk haplotype (HapA) of *ALOX5AP*. However, we found a significant association with 1 of the single markers (SG13S114) constituting this haplotype. Also, association between SG13S114 and stroke was stronger in males than in females, which is similar to the findings from Iceland. Finally, several other markers not contained in HapA showed a nominally significant association with stroke. Together, these findings suggest a weak but significant association between sequence variants in *ALOX5AP* and stroke in the present sample from central Europe.

The observed lack of association with HapA may relate in part to population differences in allele and haplotype frequencies. In support of this, the frequency of HapA was much higher in our continental sample of control individuals than in the Icelandic controls. Another explanation are different patterns of association of the high-risk allele with marker alleles and haplotypes.^{17–19} In fact, in the original study, haplotypes associated with MI were found to differ between the Icelandic and British population.³ However, interestingly, all haplotypes that had shown significant association contained either the T allele of SG13S114 or the A allele of SG13S100, both of which showed nominally significant P values in the current study. Further studies are needed to identify the causative sequence variant or variants in *ALOX5AP*.

The initial study provided data on an overall sample of stroke patients without detailed specification of stroke phenotypes. In the current study, we also looked at stroke subtypes but found no association with particular stroke etiologies. This observation suggests that the associations in the overall group are not related to associations with a single stroke subtype. Future studies with larger subgroups may specify the role of *ALOX5AP* in different stroke subtypes.

In the current study, no significant association was found between single markers or specific haplotypes of *PDE4D* and stroke. This applies to all subgroups including carotid stroke, cardiogenic stroke, and the combined carotid and cardiogenic stroke subtypes, which had shown the strongest associations in the Icelandic population. Assuming a multiplicative model and the relative risks of single markers and haplotypes reported by DeCode, the power of our sample to detect the

TABLE 4. Association of *PDE4D* Haplotypes Reported Previously to be Associated With Combined Carotid and Cardiogenic Stroke

Haplotype	German Sample			Icelandic Sample ³		
	% Cases	% Controls	P Value	% Cases	% Controls	P Value
AX*	14.3	13.7	NS	14.1	21.1	<0.01
GX*	56.8	56.5	NS	50.6	53.4	<0.001
G0	28.9	29.9	NS	35.3	25.5	<0.001

*X designates the joint set of alleles, excluding the original at-risk allele O of microsatellite AC008818-1.² Therefore, GX haplotype is the composite of all haplotypes including the G allele of SNP 45, except for the G0 haplotype and AX haplotype, including the A allele of SNP 45. Haplotype A0 does not exist. In the study by Gretarsdottir et al, GX was wild-type, AX was protective, and G0 was the at-risk haplotype.

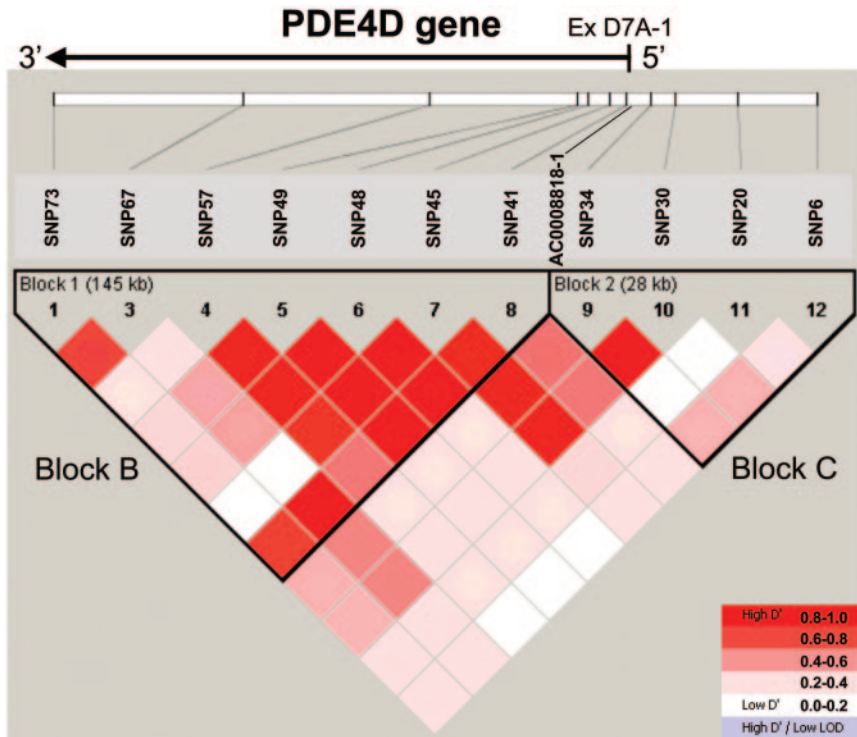


Figure 2. Pairwise LD between SNPs in the *PDE4D* gene region. A color-coded scale for D' (a measure of LD strength) is provided.

same associations should be >99% (data not shown). Also, phenotyping of our patients was particularly careful because all cases were recruited from a single unit specialized in stroke care and because diagnostic classification was based on extensive investigations with a high proportion of Doppler sonography, ECG, and MRI performed. Thus, we conclude that the *PDE4D* gene is probably not a major risk factor for overall stroke or stroke subtypes in our population.

Like the data from DeCode, our findings need to be interpreted cautiously. Our analysis of the *PDE4D* gene was limited to single markers and htSNPs from the 5' region of the gene. Thus, we cannot exclude the possibility of association with sequence variants in other regions of the *PDE4D* gene. However, we consider this possibility unlikely because most markers that had shown significant associations in the Icelandic sample were located in the 5' region of the gene.² In fact, the most significant associations had been on the very 5' end of *PDE4D*, which was covered in the current study. Another possible limitation is the low number of markers used to characterize *PDE4D*. Our set of htSNPs markers was designed to distinguish 95% of all haplotypes reported by DeCode. However, we found that the LD between single markers was less pronounced in our population, and the LD block structure differed slightly from that reported from Iceland. Thus, our set of htSNPs may have covered too little of the genetic variability in *PDE4D* in our population. On the other hand, the higher LD reported by DeCode might well reflect a founder effect in the Icelandic population, which, as a relatively isolated population, has a restricted gene pool. In fact, a founder effect in Iceland could explain the initial association and the current lack of replication in an independent population.

In conclusion, the present study suggests that sequence variants in *ALOX5AP* are in fact a significant risk factor for

stroke, particularly in males. In contrast, the *PDE4D* gene is no major risk factor for stroke in the central European population. Our findings emphasize differences in allele and haplotype frequencies as well as LD structure between populations which may account in part for the different association findings between studies.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft (KFO 113), the German Federal Ministry of Education, Science, Research, and Technology, the Estonian Science Foundation (grant no. 4578), and the State of Bavaria. The authors thank all subjects who participated in this study, T. Illig for selecting the control sample, and J. Benson for critically reading this manuscript.

References

1. Gretarsdottir S, Sveinbjornsdottir S, Jonsson HH, Jakobsson F, Einarsson E, Agnarsson U, Shkolny D, Einarsson G, Gudjonsson HM, Valdimarsson EM, Einarsson OB, Thorgeirsson G, Hadzic R, Jonsdottir S, Reynisdottir ST, Bjarnadottir SM, Gudmundsdottir T, Gudlaugsdottir GJ, Gill R, Lindpaintner K, Sainz J, Hannesson HH, Sigurdsson GT, Frigge ML, Kong A, Gudnason V, Stefansson K, Gulcher JR. Localization of a susceptibility gene for common forms of stroke to 5q12. *Am J Hum Genet.* 2002;70:593–603.
2. Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsson HM, Hawkins M, Gudmundsson G, Gudmundsdottir H, Andarson H, Gudmundsdottir AS, Sigurdardottir M, Chou TT, Nahmias J, Goss S, Sveinbjornsdottir S, Valdimarsson EM, Jakobsson F, Agnarsson U, Gudnason V, Thorgeirsson G, Fingerle J, Gurney M, Gudbjartsson D, Frigge ML, Kong A, Stefansson K, Gulcher JR. The gene encoding phosphodiesterase 4d confers risk of ischemic stroke. *Nat Genet.* 2003;35:131–138.
3. Helgadóttir A, Manolescu A, Thorleifsson G, Gretarsdottir S, Jonsdottir H, Thorsteinsdottir U, Samani NJ, Gudmundsson G, Grant SF, Thorgeirsson G, Sveinbjornsdottir S, Valdimarsson EM, Matthiasson SE, Johannsson H, Gudmundsdottir O, Gurney ME, Sainz J, Thorhallsdottir M, Andresdottir M, Frigge ML, Topol EJ, Kong A, Gudnason V, Hakonarson H, Gulcher JR, Stefansson K. The gene encoding 5-lipoxy-

- genase activating protein confers risk of myocardial infarction and stroke. *Nat Genet.* 2004;36:233–239.
4. Dixon WT, Demetrick DJ, Ohyama K, Sikora LK, Jerry LM. Biosynthesis, glycosylation and intracellular processing of the neuroglanular antigen, a human melanoma-associated antigen. *Cancer Res.* 1990;50:4557–4565.
 5. Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science.* 1983;220:568–575.
 6. Mehrabian M, Allayee H, Wong J, Shi W, Wang XP, Shaposhnik Z, Funk CD, Lusis AJ, Shih W. Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res.* 2002;91:120–126.
 7. Spanbroek R, Grabner R, Lotzer K, Hildner M, Urbach A, Ruhling K, Moos MP, Kaiser B, Cohnert TU, Wahlers T, Zieske A, Plenz G, Robenek H, Salbach P, Kuhn H, Radmark O, Samuelsson B, Habenicht AJ. Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. *Proc Natl Acad Sci USA.* 2003;100:1238–1243.
 8. Baillie G, MacKenzie SJ, Houslay MD. Phorbol 12-myristate 13-acetate triggers the protein kinase a-mediated phosphorylation and activation of the pde4d5 cAMP phosphodiesterase in human aortic smooth muscle cells through a route involving extracellular signal regulated kinase (ERK). *Mol Pharmacol.* 2001;60:1100–1111.
 9. Liu H, Palmer D, Jimmo SL, Tilley DG, Dunkerley HA, Pang SC, Maurice DH. Expression of phosphodiesterase 4d (PDE4D) is regulated by both the cyclic AMP-dependent protein kinase and mitogen-activated protein kinase signaling pathways. A potential mechanism allowing for the coordinated regulation of pde4d activity and expression in cells. *J Biol Chem.* 2000;275:26615–26624.
 10. Landells LJ, Szilagy CM, Jones NA, Banner KH, Allen JM, Doherty A, O'Connor BJ, Spina D, Page CP. Identification and quantification of phosphodiesterase 4 subtypes in cd4 and cd8 lymphocytes from healthy and asthmatic subjects. *Br J Pharmacol.* 2001;133:722–729.
 11. Indolfi C, Avvedimento EV, Di Lorenzo E, Esposito G, Rapacciuolo A, Giuliano P, Grieco D, Cavuto L, Stingone AM, Ciullo I, Condorelli G, Chiariello M. Activation of cAMP-PKA signaling in vivo inhibits smooth muscle cell proliferation induced by vascular injury. *Nat Med.* 1997;3:775–779.
 12. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE III. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* 1993;24:35–41.
 13. Ke X, Cardon LR. Efficient selective screening of haplotype tag snps. *Bioinformatics.* 2003;19:287–288.
 14. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet.* 2001;68:978–989.
 15. Zaykin DV, Westfall PH, Young SS, Karnoub MA, Wagner MJ, Ehm MG. Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. *Hum Hered.* 2002;53:79–91.
 16. Freidlin B, Zheng G, Li Z, Gastwirth JL. Trend tests for case-control studies of genetic markers: power, sample size and robustness. *Hum Hered.* 2002;53:146–152.
 17. Pritchard JK, Cox NJ. The allelic architecture of human disease genes: common disease-common variant. . . or not? *Hum Mol Genet.* 2002;11:2417–2423.
 18. Clark AG. Finding genes underlying risk of complex disease by linkage disequilibrium mapping. *Curr Opin Genet Dev.* 2003;13:296–302.
 19. Botstein D, Risch N. Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nat Genet.* 2003;33(suppl):228–237.

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Stroke. 2005;36:731-736; originally published online February 24, 2005;
doi: 10.1161/01.STR.0000157587.59821.87

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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