Variation in the PDE4D Gene and Ischemic Stroke Risk
A Systematic Review and Meta-analysis on 5200 Cases and 6600 Controls

Steve Bevan, BSc, PhD; Martin Dichgans, MD; Andreas Gschwendtner, MD; Gregor Kuhlenbäumer, MD; E.B. Ringelstein, MD; Hugh S. Markus, DM, FRCP

Background and Purpose—PDE4D was identified as the first novel gene associated with ischemic stroke risk. Replication studies have produced conflicting results, but many have been small and underpowered. Meta-analysis provides a method to combine this data and determine in a larger sample size whether the association with PDE4D can be replicated.

Methods—A meta-analysis of all PDE4D variants investigated in relation to ischemic stroke has been undertaken. Analysis of any variant appearing in 2 or more replication studies was included; this comprised 6 single nucleotide polymorphisms together with allele 0 of minisatellite AC008818 and the G0 haplotype. A total of 16 studies were identified, allowing examination of up to 5216 cases and 6615 controls for a single variant. Analyses were performed including all data, excluding data from the original report (providing true replication data), and for individual stroke subtypes and limited to white ethnicity.

Results—No individual single nucleotide polymorphism was associated with all ischemic stroke cases. Allele 0 of AC008818 and haplotype G0 carriers was associated with increased risk (relative risk, 1.12; 95% CI, 1.01 to 1.25; P=0.03 and relative risk, 1.18; 95% CI, 1.05 to 1.33; P=0.007), but these associations became nonsignificant after exclusion of the original study from the analysis (relative risk, 1.06; 95% CI, 0.94 to 1.20; P=0.34 and relative risk, 1.16; 95% CI, 1.00 to 1.34; P=0.06, respectively). Analyzing only whites, the majority of cases studied, did not result in a significant association for any analysis. Few robust associations were found with individual stroke subtypes.

Conclusion—No genetic variant examined in PDE4D showed a robust and reproducible association to ischemic stroke. Any association that may exist is likely to be weak and potentially restricted to specific populations. (Stroke. 2008;39:1966-1971.)

Key Words: genetics • meta-analysis • PDE4D

Stroke is the second largest cause of death in the Western world and a major burden on health care. Presenting in 80% of cases as ischemic stroke and 20% of cases as hemorrhagic stroke, these can be further subdivided on the basis of pathophysiology to form distinct stroke subtypes, each with different risk factors and etiologies.

Although experimental evidence suggests that genetics contributes a large part to stroke risk, until recently, the search for causative genetic factors has been largely unsuccessful.1 The identification of phosphodiesterase 4D (PDE4D) therefore caused a great deal of interest in the stroke genetics community.2 An at-risk haplotype present in 8.8% of controls was found that conferred a relative risk (RR) of ischemic stroke of 1.98, whereas a second protective haplotype was associated with a reduced risk of 0.68 relative to the wild type. An association was found with large vessel disease and cardioembolic stroke but not with small vessel disease, suggesting a possible mechanism through atherosclerosis. In addition, a dysregulation of PDE4D isoform expression was observed, providing functional evidence of a role for PDE4D in ischemic stroke risk.

The original report has been followed by many publications in an attempt to replicate this finding; some confirmed the association,3–11,20 whereas others failed to replicate it.12–16 Many of these used relatively small sample sizes, and such studies may have missed true associations of modest effect. Interpretation is further complicated by a variation in single nucleotide polymorphisms (SNPs) and markers studied by different investigators. Meta-analysis has been successfully used to bring together data from smaller inconclusive studies looking at other candidate genes in stroke.17 The large number of studies now published on PDE4D make this approach feasible. We report a systematic review and meta-analysis of the available PDE4D literature to date and include all variants that have been reported in at least 2 replication studies after PDE4D was originally identified as a predisposition gene for ischemic stroke.
Table 1. Summary of All Available Cases and Controls From Identified Studies Included in the Meta-Analysis

<table>
<thead>
<tr>
<th>Variant of All Cases</th>
<th>Identifier</th>
<th>No. of Studies</th>
<th>Total Cases</th>
<th>Total Controls</th>
<th>White Cases</th>
<th>White Controls</th>
<th>Phenotypes</th>
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<td>5</td>
<td>2598</td>
<td>3119</td>
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<td>SNP 45</td>
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<td>12</td>
<td>5216</td>
<td>6615</td>
<td>5018</td>
<td>6474</td>
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<tr>
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<td>3039</td>
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<td>3565</td>
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<td>SNP 83</td>
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<td>3498</td>
<td>2509</td>
<td>2764</td>
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<td>4442</td>
<td>3381</td>
<td>4168</td>
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<tr>
<td>SNP 89</td>
<td>rs1396476</td>
<td>5</td>
<td>2399</td>
<td>2638</td>
<td>2204</td>
<td>2489</td>
<td>All, SVD, LVD, CE, LVD+CE</td>
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<td>AC008818-1</td>
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<td>2685</td>
<td>2276</td>
<td>2685</td>
<td>All ischemic stroke only</td>
</tr>
</tbody>
</table>

*Full subtype information is available in online Supplemental Tables 1 and 2. Data are presented for all cases and white only cases. For SNP56, AC008818-1 and the G0 haplotype non-white data sets were identified.

Methods

Systematic Review

A systematic review was undertaken using the MEDLINE and PUBMED databases from 1966 onward searched on October 1, 2007. The key words stroke, SNP polymorphism, PDE4D, and phosphodiesterase 4D were searched in isolation and in combination with each other. From an initial 119,426 references in isolation, combination searches produced 24 unique references, including reviews and editorials. These were then hand-searched by 2 independent researchers for novel references and for data that could be included in a meta-analysis; no additional references were found. Of the 24 references identified, 16 contained relevant genetic association data suitable for inclusion.2,16,20

The criteria for a genetic variant (SNP or minisatellite) to be included in the meta-analysis was that it must have been included in at least 2 published studies after the original identification of PDE4D in ischemic stroke. This identified 7 SNPs, although a subsequent misclassification of the rs number for SNP 41 in the original paper of Gretarsdottir et al.,18 and continuation of this error in subsequent studies, meant this variant was excluded from further analysis to leave 6 SNPs. The 6 SNPs were 26, 45, 56, 83, 87, and 89 (variant numbering from the original publication has been maintained for consistency). In addition, it was possible to perform a meta-analysis on carriers of allele 0 of minisatellite AC008818-1 and haplotypes of this minisatellite with SNP 45, the at-risk haplotype originally described from the G0 haplotype no non-white data sets were identified.

Meta-Analysis

Raw data were extracted from papers where possible with frequencies multiplied by the given sample size where necessary. For the data set reported in Lohmusar et al.,15 additional genotyping was performed by the original authors for variants SNP 26 and SNP 87 using similar methods to those described in the original paper, and this was included in the meta-analysis. Where data were not available for extraction from published articles, it was sought and provided by personal communication (from S. Gretarsdottir2 and K. Kostulas15). Full details of the data extracted from the 16 reported papers are available as supplementary online material (supplemental Table II, available online at http://stroke.ahajournals.org ). Meta-analysis was performed using Review Manager (RevMan) version 4.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2006; www.cc-ims.net/RevMan).

A total of 7 references reported stroke subtype information and were included in the meta-analysis.2,10–15 Of these, 5 used TOAST criteria to assign subtypes. Woo et al11 used stroke subtype definitions adapted from the Classification of Cerebrovascular Diseases III and epidemiological studies performed in Rochester Minnesota (full details available at http://stroke.ahajournals.org/cgi/content/full/37/2/371). These are similar to those used in the TOAST classification. van Rijn et al10 used a similar definition to the TOAST for small vessel disease: an appropriate infarct <1.5 cm detectable on CT or MRI or, in the absence of CT infarction, a clinical lacunar syndrome with no evidence of cortical dysfunction. They used a definition of large artery disease that did not include arterial imaging and that is likely to have included cases of both cardioembolic stroke and stenotic large artery disease; they classified a large vessel infarction if a lesion on CT or MRI was >1.5 cm or clinical findings included cortical impairment or brainstem or cerebellar dysfunction. Due to this difference in subtype classification, this study was not used in subtype analysis.

Results

A summary of the data identified from the systematic review is shown in Table 1 for each of the 6 SNPs. Table 1 also shows the same information for minisatellite AC008818-1 and haplotype G0 formed from allele G of SNP 45 and allele 0 of the minisatellite. Full details of all the data extracted by publication, variant, and phenotype are available online in supplemental Table II. Of the 6 SNPs, SNP 45 has been most extensively replicated, appearing in 12 publications examining a total of 5216 cases and 6615 controls. Data were available on at least 2260 cases and 2600 controls for all of the SNPs, the minisatellite, and the G0 haplotype.

A stepwise series of analyses was performed. Initially, meta-analysis of all stroke subtypes was performed including data from the original report.2 This was followed by analysis of the original report, providing a true test of replication. Further analysis was performed of associations with individual stroke subtypes, both including and excluding the original report. Analyses were also performed limited to whites.

Initial analysis combined all cases and controls, including those from the original study of Gretarsdottir et al.,2 irrespective of ethnicity, sex, and stroke subtype. The results of this are detailed in Table 2 and supplemental Table I. No single SNP alone was identified as having a significant association with ischemic stroke when examined as both carriers and homozygotes for the SNP in question. Carriers of allele 0 of AC008818 showed a significant association with stroke risk in all stroke cases (RR, 1.12; 95% CI, 1.01 to 1.25; z=2.20; P=0.03) and this risk increased for carriers of the G0 haplotype of SNP 45 and allele 0 of AC008818 (RR, 1.18; 95% CI, 1.05 to 1.33; z=2.68; P=0.007). Lack of sample numbers precluded an analysis of individuals possessing 2 copies of either allele 0 alone or the G0 haplotype. Forrest plots for these analyses are shown in Figure.

This analysis was then repeated with exclusion of data from the original report by Gretarsdottir et al.2 Again, there
was no association with any individual SNP. In contrast to the data including all studies, the presence of allele 0 of AC008818 no longer showed a significant association. Carriers of haplotype G0, which was significantly associated with ischemic stroke when the original study was included, just failed to reach significance (RR, 1.16; 95% CI, 1.00 to 1.34; z = 1.91; P = 0.05). When the same analyses were repeated with the exclusion of the original study, both became nonsignificant (RR, 1.26; 95% CI, 1.09 to 1.42; z = 2.07; P = 0.04). Exclusion of the original study of Gretarsdottir et al from these white-specific analyses again resulted in all significant associations between SNP 45 on the combined subtype of LVD+CE (RR, 0.84; 95% CI, 0.70 to 1.00; z = 1.96; P = 0.05), and SNP 89 was associated with reduced risk of SVD (RR, 0.79; 95% CI, 0.62 to 1.00; z = 1.98; P = 0.05). When the same analyses were repeated with the exclusion of the original study, both became nonsignificant (RR, 0.93; 95% CI, 0.76 to 1.14; z = 0.67; P = 0.50 and RR, 0.84; 95% CI, 0.64 to 1.10; z = 1.26; P = 0.21, respectively; supplemental Table I).

Table of associations with stroke subtypes was repeated restricted to whites. SNP 56 remained significantly associated with cardioembolic stroke in whites (RR, 0.82; 95% CI, 0.69 to 0.92; z = 2.13; P = 0.03), whereas the association between SNP 89 and SVD was lost. Two new associations were also identified with stroke subtype in whites only: presence of SNP 45 on the combined subtype of LVD+CE (RR, 0.89; 95% CI, 0.79 to 1.00; z = 2.04; P = 0.04) and SNP 56 in the same combined subtype of LVD+CE (RR, 0.86; 95% CI, 0.75 to 0.99; z = 2.07; P = 0.04; Table 2). Exclusion of the original study of Gretarsdottir et al from these white-specific analyses again resulted in all significant associations between

### Table 2. Association Between All Variants and all Ischemic Stroke Together With Significant Associations With Ischemic Stroke Subtypes*

<table>
<thead>
<tr>
<th>Analysis</th>
<th>No. of Studies</th>
<th>No. of Cases</th>
<th>No. of Controls</th>
<th>OR</th>
<th>95% CI</th>
<th>Z Score</th>
<th>Significance</th>
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<tr>
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<td>6</td>
<td>1752/2638</td>
<td>2013/3119</td>
<td>0.96</td>
<td>0.86–1.07</td>
<td>0.79</td>
<td>0.43</td>
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<tr>
<td>SNP 26 homozygotes</td>
<td>6</td>
<td>462/2638</td>
<td>538/3119</td>
<td>1.04</td>
<td>0.90–1.19</td>
<td>0.50</td>
<td>0.62</td>
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<td>SNP 45 carriers</td>
<td>13</td>
<td>1571/5216</td>
<td>1913/6615</td>
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<td>0.91–1.07</td>
<td>0.24</td>
<td>0.81</td>
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<tr>
<td>SNP 45 homozygotes</td>
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<td>157/5216</td>
<td>180/6615</td>
<td>1.00</td>
<td>0.81–1.25</td>
<td>0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>SNP 56 carriers</td>
<td>7</td>
<td>1692/3039</td>
<td>2043/3684</td>
<td>0.98</td>
<td>0.89–1.08</td>
<td>0.43</td>
<td>0.66</td>
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<td>SNP 56 homozygotes</td>
<td>7</td>
<td>396/3039</td>
<td>432/3684</td>
<td>1.06</td>
<td>0.91–1.23</td>
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<td>2181/3498</td>
<td>0.96</td>
<td>0.87–1.07</td>
<td>0.73</td>
<td>0.46</td>
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<td>612/3498</td>
<td>0.97</td>
<td>0.85–1.10</td>
<td>0.51</td>
<td>0.61</td>
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<tr>
<td>SNP 87 carriers</td>
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<td>2582/3653</td>
<td>3154/4442</td>
<td>0.98</td>
<td>0.89–1.08</td>
<td>0.34</td>
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<td>0.89–1.08</td>
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<td>0.73</td>
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<td>698/2399</td>
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<td>0.95</td>
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<td>1284/4468</td>
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<td>0.79–1.00</td>
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<td>0.70–1.00</td>
<td>1.96</td>
<td>0.05**</td>
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<tr>
<td>SNP 89 SVD carriers</td>
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<td>108/421</td>
<td>696/2288</td>
<td>0.79</td>
<td>0.62–1.00</td>
<td>1.98</td>
<td>0.05**</td>
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<tr>
<td>SNP 56 CE carriers white</td>
<td>3</td>
<td>296/599</td>
<td>1293/2381</td>
<td>0.82</td>
<td>0.69–0.98</td>
<td>2.13</td>
<td>0.03**</td>
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<tr>
<td>SNP56 LVD+CE carriers white</td>
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<td>616/1219</td>
<td>1293/2381</td>
<td>0.86</td>
<td>0.75–0.99</td>
<td>2.07</td>
<td>0.04**</td>
</tr>
</tbody>
</table>

*Significant associations with ischemic stroke subtypes are shown for both all cases together and white-only cases.

**Significance at P = 0.05.
ischemic stroke subtype and genotype being lost (supplemental Table I).

Discussion

Since the initial identification of PDE4D as a risk gene for ischemic stroke, there have been numerous replication studies in a number of populations with conflicting results. Although the findings of Gretarsdottir et al identified a maximal risk with a haplotype comprising a number of SNPs and a single allele of a minisatellite, to date, the majority of the replication studies have been less intensive and concentrated on a subset of variants originally studied by the DeCode group. However, the considerable amount of replication data published, in more than 5200 cases and 6600 controls, is sufficient to allow meta-analysis of the PDE4D gene region and its involvement in ischemic stroke. This approach overcomes a major limitation of the published replication data, namely that sample sizes have usually been too small to definitively confirm or refute replication.

In this meta-analysis, no individual SNP of the 6 investigated showed a significant association with ischemic stroke. An analysis of carriers of allele 0 of AC008818 and carriers of the G0 haplotype in this group did reveal a significant association in both instances. A secondary analysis on the same data excluding the original study identifying PDE4D was then conducted to assess the true “replication” effect of the subsequent studies. No individual SNP was significantly associated with ischemic stroke in this analysis. With the exclusion of the original study, however, both allele 0 of AC008818 and haplotype G0 also became negative and showed no association with ischemic stroke.

In an effort to ensure population stratification was not having an adverse effect on the meta-analysis through ethnicity, these analyses were repeated again in white subjects only. In both all white cases and all white cases with the exclusion of the original study, no individual SNP was significantly associated with ischemic stroke. The association with allele 0 of AC008818 and haplotype G0 remained when the original study when was included, because no nonwhite populations were typed for these variants, but disappeared when data from the original study were excluded.

Although the original investigation of Gretarsdottir et al identified PDE4D as a risk in all ischemic stroke, risks were greater in certain ischemic stroke subtypes, in particular in the combined subtype of cardioembolic and large artery disease. Therefore, meta-analysis was repeated in individual subtypes where data were available. Few associations were reported with specific subtypes. SNP 56 was associated with CE stroke, SNP 45 with the combined LVD+CE subtype, and SNP 89 with SVD stroke. With exclusion of the original study, however, these all became nonsignificant. When restricting subtype analysis to white ethnicity, SNP 56 remained significant in CE stroke and became positive in the combined LVD+CE subtype. Again, however, on exclusion of the original data, both of these associations were lost.

Taken together, these data fail to show a replication of the association between PDE4D and ischemic stroke. Indeed, exclusion of the original study resulted in no significant
associations from the combined replication studies for any SNP or other variant in all ischemic stroke or any stroke subtype.

An important consideration in any meta-analysis is that of heterogeneity between studies as a result of publication bias, small sample size, a small number of studies, or comparison of disparate phenotypes. Examination of heterogeneity in this study (see supplemental Table I for heterogeneity scores) shows 32 of the 198 analyses conducted contained significant heterogeneity as measured by \( \chi^2 \) and assuming a fixed effect model. Although this at first seems a high proportion of studies, further examination shows of those 32 studies, only 6 show heterogeneity when the original study of Gretarsdottir et al is excluded. This is perhaps not surprising given the failure to replicate these findings in subsequent individual replication studies; inclusion of a highly significant study among a series of nonsignificant studies is likely to induce heterogeneity into the meta-analysis. Furthermore, of the 6 true replication analyses showing heterogeneity, 2 are in SNP 83 carriers for all cases and carriers for all white samples, the remaining 4 are also “duplicated” being in SNP 87 LVD carriers and CE homozygotes in all cases and white cases. None of these 6 comparisons show an overall significant association between genotype and phenotype.

The only previous attempt to examine systematically the role in PDE4D in ischemic stroke did not perform meta-analysis, because insufficient data were available at that time.\(^{19}\) It examined all 11 reported nominally significant replicated SNPs in PDE4D and examined their correlation with the at-risk haplotype G0 in the CEPH trios from the Hapmap project. They found that no SNP was identified that correlated strongly with the G0 haplotype and concluded that subsequently reported associations were unlikely to be true replication given this lack of correlation. Since this analysis, there have been a number of further replication studies now providing sufficient data for a meta-analysis. The results of our meta-analysis reported here agree entirely with the earlier conclusion.

The size of the populations available allows an estimate of the effect size excluded by a negative meta-analysis. SNP 45 represented the most well replicated SNP, comprising 5216 cases and 6615 controls. With a sample this large, we had greater than 99% power to detect a risk of 1.2 and greater than 94% power to detect a risk of 1.15. With the least investigated SNP, SNP 26, which has 2598 cases and 3119 controls, we have greater than 99% power to detect a risk of 1.3 and greater than 89% power to detect a risk of 1.2. These calculations take a minor allele frequency of 0.2 (equivalent to that of both SNP 45 and SNP 26) and a significance level of 0.05 because each is testing a single hypothesis.

Although we were able to include data from over 11 000 cases and controls in this meta-analysis, there are still some limitations to these results. Most of the replication studies investigated relatively few variants and fewer than those described in the original publication. Not all studies included a sufficient degree of stroke subtyping to allow analysis of their data for associations with specific subtypes. This is a major limitation of genetic replication studies in general and one that needs to be considered when replicating future reported associations. As a minimum, all significantly associated variants in an original report need to be included in any true replication study. In addition, replication populations should be phenotypically matched to the originally reported population if at all possible, particularly where associations are reported with defined stroke subtypes. The lack of replication studies examining allele 0 of AC008818 has proved to be a major limitation in truly assessing the effect of PDE4D on stroke by meta-analysis.

In addition to differentially analyzed variants, differences in population substructure may complicate interpretation; this is a particular issue because the underlying functional mutations in PDE4D associated with the increased stroke risk have not been identified. Publication bias is an important potential confounding factor in a meta-analysis; however, this tends to exaggerate any association and would not be expected to result in a lack of association.

In conclusion, although meta-analysis of an association with the PDE4D gene region and ischemic stroke revealed several significant associations, most notably with the G0 haplotype, all became nonsignificant with the exclusion of the original study. Therefore, this study does not provide support for the association by replication in other studies. Although the data presented here cannot totally exclude a link between PDE4D and ischemic stroke, failure to show any replication of the original investigation when considering all replication studies published to date suggests that any association that may exist is likely to be weak and possibly restricted to specific populations.

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Disclosures
None.

References


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