The Genetics of Cervical Artery Dissection: A Systematic Review
Stéphanie Debette and Hugh S. Markus
Stroke 2009;40:e459-e466; originally published online Apr 23, 2009;
DOI: 10.1161/STROKEAHA.108.534669
Stroke is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
Copyright © 2009 American Heart Association. All rights reserved. Print ISSN: 0039-2499. Online
ISSN: 1524-4628

The online version of this article, along with updated information and services, is
located on the World Wide Web at:
http://stroke.ahajournals.org/cgi/content/full/40/6/e459
Background and Purpose—The pathophysiology of cervical artery dissections (CAD), a major cause of ischemic stroke in young adults, is poorly understood. Several arguments suggest a genetic predisposition.

Methods—We systematically reviewed all published data on genetic risk factors for CAD and performed a meta-analysis of association studies with the \textit{MTHFR} C677T polymorphism.

Results—Rarely, CAD is associated with a known monogenic connective tissue disease, mainly vascular Ehlers-Danlos syndrome. However, in the large majority of CAD cases, there is no evidence for a known monogenic disease. Several arguments, including the association of CAD with dermal connective tissue abnormalities that are inherited, suggest that genetic factors also play a role in “sporadic” CAD as part of a multifactorial predisposition. We identified 15 genetic association studies: 10 were negative and 5 reported associations of 3 genetic variants in 3 different candidate genes. Two studies reported associations with polymorphisms in ICAM-1 and COL3A1, but neither has been replicated. Three studies reported an association with the \textit{MTHFR} 677TT genotype, but 3 other studies did not replicate this. A meta-analysis of these data identified an overall significant association of the \textit{MTHFR} 677TT genotype with CAD (OR, 1.67; 95% CI, 1.21 to 2.31). We also identified 9 studies screening candidate genes for mutations and 4 linkage studies, yielding mostly negative results.

Conclusions—Although several interesting hypotheses were generated, the majority of genetic studies in CAD have been negative until now, but they were markedly underpowered. Progress in unraveling the genetics of CAD will require the collection of DNA samples from large multicenter series. (\textit{Stroke}. 2009;40:e459-e466.)

Key Words: dissection \mismatch genetics \mismatch carotid artery \mismatch vertebral artery \mismatch stroke

Cervical artery dissections (CADs) are a common cause of ischemic stroke in young adults.\cite{footnote} The incidence of CAD is estimated at 2.6 to 2.9 per 100,000 per year in the general population,\cite{footnote2} and the mean age of occurrence is 44 to 46 years.\cite{footnote3}

The pathophysiology of CAD is poorly understood. It has been associated with major head and neck trauma\cite{footnote4} as well as with minor trauma secondary to a wide range of insults.\cite{footnote5} Other risk factors proposed include recent infection,\cite{footnote6} hyperhomocysteinemia,\cite{footnote7}–\cite{footnote9} migraine,\cite{footnote10} low levels of $\alpha_1$-antitrypsin,\cite{footnote11} and hypertension\cite{footnote12}–\cite{footnote14} and fibromuscular dysplasia,\cite{footnote15} but most evidence is limited.\cite{footnote16}

Several arguments suggest genetic factors may play an important role in the pathophysiology of CAD, in rare cases as part of a single gene disorder and more commonly as part of a multifactorial predisposition (Figure 1).\cite{footnote17} It has been hypothesized that patients with CAD could have a constitutional, genetically determined weakness of the vessel wall and that environmental factors such as acute infection or minor trauma could act as triggers.\cite{footnote18} Genetic factors could also contribute to CAD occurrence at other levels, eg, through predisposition to inflammation and thrombosis (Figure 1).

We have performed a systematic review of published data on genetic risk factors of CAD.

Search Strategy and Selection Criteria

References for this review were identified through searches of PubMed from 1966 to May 1, 2008, with the terms “carotid artery, internal,” “carotid arteries,” “vertebral artery,” “dissection,” “gene,” “genotype,” “alleles,” “polymorphism, genetic,” “haplotypes,” “genetic markers,” “linkage,” “mutation,” “sequence analysis, DNA,” “Ehlers-Danlos syndrome,” “marfan syndrome,” “cutis laxa,” “pseudoxanthoma elasticum,” “loeys-dietz,” “polycystic kidney, autosomal dominant,” and “alpha 1-antitrypsin deficiency.” Reference lists of relevant articles were also reviewed.

Case reports and genetic association studies on less than 20 CAD cases, or where CAD was studied in a post hoc subgroup analysis, were not included. We also excluded studies on purely intracranial dissections, a different phenotype than CAD, although both may have some predisposing risk factors in common. Details on the reviewing procedure are provided as supplemental data.
For the MTHFR C677T polymorphism, a meta-analysis was performed using RevMan (Version 4.2) software (www.cc-ims.net/RevMan/current.htm). A fixed-effects meta-analysis using the Mantel-Haenszel method was implemented. Heterogeneity between studies was assessed by the $\chi^2$ test and the $I^2$ statistic.

**Single Gene Disorders Causing CAD**

**Vascular Ehlers-Danlos Syndrome**

Vascular Ehlers-Danlos syndrome (vEDS) is a rare autosomal-dominant disease due to a mutation in the COL3A1 gene (OMIM 13050) with a prevalence estimated at 0.2 to one per 100 000 and a median survival of 48 years. The diagnosis is suggested by the presence of 2 of 4 clinical criteria (easy bruising, thin skin with visible veins, characteristic facial features, and rupture of arteries, uterus, or intestines) and confirmed by the demonstration of abnormal Type III procollagen synthesis or a mutation in the COL3A1 gene.

In a series of 16 patients with vEDS undergoing an ultrasound protocol, one (6%) had a history of documented CAD. In a series of 31 patients with vEDS, ascertained through a vascular surgery department, 24 patients experienced 132 vascular complications, 8 in the carotid and 2 in the vertebral arteries, but it is not mentioned what proportion of these were dissections. In the 2 largest, partly overlapping, series of biologically confirmed patients with vEDS, 2% of the patients had a history of CAD, although only carotid dissections were reported in the most recent study.

The reported rate of vEDS cases in large published series of consecutive patients with CAD is very low. Among large CAD series including over 100 patients, vEDS was found in 0.5% to 2% of the patients (none of these articles mentions whether the diagnosis of vEDS was confirmed biologically). Several other large series do not report any patient with vEDS. This maybe slightly underestimated because none of the large CAD series has systematically searched for diagnostic criteria of vEDS. Overall, despite this limitation, CAD cases with vEDS seem to be very rare, representing less than 2% of all CAD cases.

**Marfan Syndrome**

Marfan syndrome (MFS) is an autosomal-dominant disease due to a mutation in the fibrillin-1 gene (OMIM 154700). The prevalence is estimated at one per 5000 individuals (www.orpha.net) and a mean survival of 45±17 years. The clinical signs in MFS are mainly musculoskeletal, ocular, cardiac with aortic and mitral valve anomalies, and aortic aneurysms and dissections.

In a retrospective analysis of neurovascular complications in 513 MFS patients, no CAD was found. Similarly, no case of CAD was reported in a recent series of 1013 patients with MFS, although central nervous system complications are not described in detail. Large series of consecutive patients with CAD report very low frequencies of MFS (0.6% to 0.9%) without details on how the diagnosis of MFS was confirmed. Thus, spontaneous CAD seems to be exceptional in patients with a proven diagnosis of MFS (and should be distinguished from proximal aortic dissections extending into the brachiocephalic arteries).

**Other Monogenic Disorders**

Aneurysms on head or neck arterial branches have been reported in patients with Loeys-Dietz syndrome, an autosomal-dominant disease caused by mutations in the TGFBR1 and TGFBR2 genes. Whether extracranial artery dissection could cause the reported cerebral aneurysms is unclear.
Evidence for an association of CAD with monogenic conditions such as α-1 antitrypsin deficiency, osteogenesis imperfecta, autosomal-dominant polycystic kidney disease, or hereditary hemochromatosis is insufficient.

Genetics of Sporadic CAD
How Important Are Genetic Factors in Apparently Sporadic CAD Cases?
In most CAD cases, there is no evidence for an underlying monogenic disease. Heritability estimates of apparently sporadic CAD are not available. Two studies on 181 and 200 patients with CAD reported the presence of a family history of CAD in 2% to 3% of their patients. This is certainly overestimated due to recruitment bias and the fact that all CAD cases from multiply affected families were included. Other large series of consecutive patients with CAD did not report any family history of CAD, but this may not have been systematically searched for. Besides, a family history of CAD is likely to be underreported because CAD can occur asymptotically and because CAD may have been unrecognized in parents of patients with CAD before MRI became widely available.

Another argument suggesting a genetic predisposition to CAD is the familial aggregation with dissections in other locations (intracranial arteries, renal arteries, and aorta). Patients with CAD also often present concomitant arterial anomalies such as fibromuscular dysplasia, aortic root dilation, hyperdissociability of the arterial wall, or endothelial dysfunction, and an association with intracranial aneurysms and temporal artery histological changes has been suggested by some authors.

Finally, important evidence supporting the role of genetic predisposition comes from the high prevalence of connective tissue abnormalities reported in skin biopsies taken from apparently sporadic CAD cases. Over 50% of these subjects had connective tissue aberrations in their reticular dermis, the most common pattern being collagen fibrils and fragmentation of elastic fibers. These abnormalities seem to be transmitted according to an autosomal-dominant pattern without fulfilling the diagnostic criteria for known monogenic connective tissue disorders.

A number of approaches have been used to determine the underlying genetic variants contributing to sporadic CAD risk, including (1) screening for monogenic causes through systematic sequencing; (2) linkage studies; and (3) genetic association studies.

Screening for Monogenic Causes of CAD Through Systematic Sequencing of Candidate Genes
Several studies have screened for mutations in different candidate genes (Table 1). They were performed on small series with, in most cases, either a family history of CAD or morphological abnormalities in dermal connective tissue.

Four studies have screened for mutations in COL3A1 (causing vEDS) in a total of 53 patients with CAD. All were negative, except for one study that found a G157S missense mutation in 2 related patients with CAD (Table 1). This type of mutation (glycine substitution in the triple helical region of COL3A1) is typical of vEDS, but both subjects had no clinical evidence of this disease.

Other studies have looked for disease-causing mutations in genes that are not known monogenic causes of CAD but are involved in connective tissue homeostasis (Table 1). The only potentially disease-causing mutation that was found, in COL5A2, was considered as a neutral variant because it does not correspond to the typical disease-causing mutations in COL5A2 and because it is in an amino acid position that was not conserved in evolution. This mutation was not found in an additional series of 50 patients with CAD. Noteworthy, a novel G213V variant was recently detected in the COL5A2 gene of a young patient with recurrent carotid artery dissections.

Linkage Studies
Linkage studies are limited by the small number of large families with several members affected by CAD. One linkage analysis was performed in a family with 3 members affected by CAD using CA repeat markers that flank the COL3A1 locus yielding negative results.

Other linkage studies have been performed in families with only one member affected by CAD but several members presenting dermal connective tissue aberrations (intermediate phenotype). A first study tested linkage with microsatellite markers for 43 candidates genes involved in the synthesis of extracellular matrix components in one family. Most genes were excluded with logarithm of odds scores < -2.0. For 3 markers, harboring 4 candidate genes (PLOD, FBLN2, COL16A1, TIMP), a cosegregation of the hypothetical disease locus and the analyzed microsatellite markers was found, but the maximum logarithm of odds score in this family was 0.9, which is insufficient to confirm the presence of genetic linkage (this requires a logarithm of odds score > 3.0). A second study tested linkage with 4 microsatellite markers flanking the COL8A1 and COL8A2 genes in one family with negative results. A third study performed a whole genome linkage analysis and identified 2 suggestive candidate loci on chromosomes 15q24 and 10q26 with logarithm of odds scores of 2.1 and 1.9 in one family. The same regions were, however, excluded in 2 other families, suggesting locus heterogeneity of the connective tissue phenotype.

Genetic Association Studies
Our systematic review identified 15 genetic association studies (Table 2). Most of these were negative. Five studies reported associations with 3 different candidate genes: ICAM-1, COL3A1, and MTHFR. The associations with the ICAM-1 E469K polymorphism and the COL3A1 3’UTR 2-bp deletion, observed in 2 relatively small studies, have not been replicated and should therefore be interpreted with caution. The ICAM-1 E469K polymorphism could modify the affinity of ICAM-1 to its ligands, which may lead to increased activation of cytokines and proteases, thus inducing extracellular matrix degradation and weakening of the arterial wall. The COL3A1 3’UTR 2-bp deletion may influence COL3A1 expression and thus extracellular matrix homeostasis.
Three studies found a positive association between the methylenetetrahydrofolate reductase (MTHFR) 677TT genotype and CAD.8,9,66 Three other studies did not find any significant association between MTHFR 677TT and CAD.7,13,60

We performed a meta-analysis of 5 studies7,9,13,60,66 (in 440 cases and 1220 control subjects); the sixth study8 was not included in the meta-analysis because of overlapping data with a subsequent analysis from the same group.66 The meta-analysis showed an overall significant association of the MTHFR 677TT genotype with CAD with an OR of 1.67 (95% CI 1.21 to 2.31; Figure 2). There was no significant heterogeneity between studies ($I^2 = 33.9\%$, $P = 0.20$), although the power to detect heterogeneity was low given the small number of studies and their small size. A meta-analysis using a random-effects model was also performed and yielded similar results (data not shown). These results support a modest association between the MTHFR 677TT genotype and CAD. However, given the potential publication bias favoring results that show an association, it is important to replicate this finding in a larger, independent sample of patients with CAD. The MTHFR 677TT genotype is associated with elevated homocysteine levels.7–9,13 Elevated homocysteine levels could contribute to CAD by endothelial damage or by elevated homocysteine levels.7–9,13

### Future Directions

Although some results have generated interesting hypotheses, previous genetic association studies on CAD have been mostly negative. However, these studies have been markedly underpowered, mainly due to the low prevalence of CAD, which made it difficult to reach sufficient sample sizes; none...
Table 2. Candidate Genes for Which Associations With CAD Have Been Examined

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Polymorphism</th>
<th>Study Size</th>
<th>Population</th>
<th>Associated Allele/Genotype, P Value, OR</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td>1p36.3</td>
<td>C677T (rs1801133)</td>
<td>26 cases, 30 controls</td>
<td>Italian</td>
<td>NS</td>
<td>Gallai, 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>95 cases, 95 controls</td>
<td>German</td>
<td>NS†</td>
<td>Konrad, 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(I) 25 cases, 36 controls; (II) 25 cases, 31 subjects with IS*</td>
<td>174 cases, 927 controls</td>
<td>Italian</td>
<td>(I) TT more frequent in cases, P=0.045; (II) NS</td>
<td>Pezzini, 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>39 cases, 76 controls</td>
<td>German</td>
<td>NS‡</td>
<td>Kloss, 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(I) 106 cases, 187 controls; (II) 106 cases; 227 subjects with IS*</td>
<td>95 cases, 95 controls</td>
<td>German</td>
<td>(I) OR, 2.56 (95% CI, 1.43–4.38) for TT carriers; (II) NS</td>
<td>Arauz, 2007</td>
</tr>
<tr>
<td>Cystathionine β-synthase (CBS)</td>
<td>21q22.3</td>
<td>844ins68bp</td>
<td>25 cases, 36 controls</td>
<td>Italian</td>
<td>(I) NS; (II) NS</td>
<td>Pezzini, 2002</td>
</tr>
<tr>
<td>Methylenetetrahydrofolate dehydrogenase1 (MTHFD1)</td>
<td>14q24</td>
<td>G1958A</td>
<td>95 cases, 95 controls</td>
<td>German</td>
<td>NS‡</td>
<td>Konrad, 2004</td>
</tr>
<tr>
<td>Intercellular adhesion molecule 1 (ICAM-1)</td>
<td>19p13.3-p13.2</td>
<td>E469K</td>
<td>96 cases, 204 controls</td>
<td>German</td>
<td>469E more frequent in cases P=0.005</td>
<td>Longoni, 2006</td>
</tr>
<tr>
<td>Collagen, Type I, α-2 (COL1A2)</td>
<td>7q22.1</td>
<td>rs42524</td>
<td>144 cases, 162 controls</td>
<td>German</td>
<td>NS§</td>
<td>Kuhlenbaumer, 2006</td>
</tr>
<tr>
<td>Collagen, Type III, α-1 (COL3A1)</td>
<td>2q31</td>
<td>Intron 24 CA repeat; intron 25 VNTR; exon 31 Alu-RFLP; 3' UTR 2-bp deletion; 3' flanking Avail-RFLP</td>
<td>45 cases, 50 controls</td>
<td>German</td>
<td>3' UTR 2-bp deletion more frequent in cases, P&lt;0.03; NS for the other polymorphisms</td>
<td>Von Pein, 2002</td>
</tr>
<tr>
<td>α-1 antitrypsin (AAT)</td>
<td>14q32.1</td>
<td>PIM, PIZ, PIS</td>
<td>74 cases, 74 controls</td>
<td>German</td>
<td>NS‡</td>
<td>Grund-Ginsbach 2004</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>7p21</td>
<td>−597G/A; −572G/C; −373 A(n)/T(n); −174G/C</td>
<td>80 cases, 80 controls</td>
<td>German</td>
<td>NS§</td>
<td>Wiest, 2004</td>
</tr>
<tr>
<td>Matrix metalloproteinase-9 (MMP-9)</td>
<td>20q11.2-q13.1</td>
<td>Promoter CA repeat −1562C/T</td>
<td>52 cases, 52 controls</td>
<td>German</td>
<td>NS‡</td>
<td>Wagner, 2004</td>
</tr>
<tr>
<td>Selenoprotein S (SEPS1)</td>
<td>15q26.3</td>
<td>rs28665122</td>
<td>260 cases, 393 controls</td>
<td>German, Italian</td>
<td>NS‡</td>
<td>Hyrenbach, 2007</td>
</tr>
<tr>
<td>Lysyl oxidase like1 (LOXL1)</td>
<td>15q22</td>
<td>rs3825942; rs893817; rs1048661; rs2165241; rs838818; rs893820; rs750460; rs2304722; rs11072450; rs3522; rs7173049; rs7175324</td>
<td>157 cases, 216 controls</td>
<td>German</td>
<td>NS§‡</td>
<td>Kuhlenbaumer, 2007</td>
</tr>
</tbody>
</table>

*IS indicates ischemic stroke of another cause than dissection.  
†The 677TT genotype was significantly more frequent (P=0.03) in the 14 patients with multiple dissections.  
‡The difference was significant when comparing the 3 genotypes using a general model.  
§This study found a borderline significant association with 2 LOXL1 single nucleotide polymorphisms (rs3825942 and rs893817) before correcting for multiple testing.  
NS indicates nonsignificant.
reached a sample size >300 cases and only 4 had >100 cases. For other complex genetic diseases, the ORs associated with individual genetic variants is almost always below 1.5. If the frequency of the allele being tested is 20%, to detect an OR of 1.5 would require 268 cases and control subjects, whereas detecting an OR of 1.3 would require 906 cases, and these sample sizes rise if the allele frequency is <20%. Therefore, definitive data will only be obtained from much larger multicenter genetic association studies such as the Cervical Artery Dissections and Ischemic Stroke Patients (CADISP) consortium (www.cadisp.org) with replication of positive associations in independent samples.

Even when larger sample sizes and more robust methodology are used, candidate gene association studies are unable to identify novel genetic variants involved in unsuspected pathways, because they are based on what is already known or suspected about the pathophysiology of the disease. Genomewide association studies offer a solution to this problem by genotyping large numbers of single nucleotide polymorphisms distributed across the chromosomes without requiring any a priori hypothesis. This approach has recently been applied to a number of complex diseases with notable successes, eg, identification of novel genes conferring increased risk of diabetes and coronary heart disease. This new approach may be equally well suited to CAD if sufficiently large populations can be collected.

Finally, although genetic association studies are generally more efficient than linkage studies in multifactorial diseases, extending the number of linkage studies on large families with inherited dermal connective tissue alterations could be useful. Candidate genes may then be selected from regions that have been identified through these linkage studies and their association with CAD could be tested in large case–control association studies.

Acknowledgments

S.D. received a grant from the European Neurological Society (2007) and is grateful for support from the Department of Neurology (EA2691) of the University Hospital of Lille, France.

Disclosures

None.

References


20. Germain DP. Ehlers-Danlos syndrome type IV.


