CADISP-genetics: an International project searching for genetic risk factors of cervical artery dissections

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Background **Cervical artery dissection (CAD) is a frequent cause of ischemic stroke, and occasionally death, in young adults. Several lines of evidence suggest a genetic predisposition to CAD. However, previous genetic studies have been inconclusive mainly due to insufficient numbers of patients. Our hypothesis is that CAD is a multifactorial disease caused by yet largely unidentified genetic variants and environmental factors, which may interact. Our aim is to identify genetic variants associated with an increased risk of CAD and possibly gene–environment interactions.**

Methods **We organized a multinational European network, *Cervical Artery Dissection and Ischemic Stroke Patients* (CADISP), which aims at increasing our knowledge of the pathophysiological mechanisms of this disease in a large group of patients. Within this network, we are aiming to perform a *de novo* genetic association analysis using both a genome-wide and a candidate gene approach. For this purpose, DNA from approximately 1100 patients with CAD, and 2000 healthy controls is being collected. In addition, detailed clinical, laboratory, diagnostic, therapeutic, and outcome data are being collected from all participants applying predefined criteria and definitions in a standardized way. We are expecting to reach the above numbers of subjects by early 2009.**

Conclusions **We present the strategy of a collaborative project searching for the genetic risk factors of CAD. The CADISP network will provide detailed and novel data on environmental risk factors and genetic susceptibility to CAD.**

Key words: dissection, genetics, ischemic stroke, polymorphisms, risk factors, SNP

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Conflict of interest: None.

Introduction

The annual incidence of cervical artery dissections (CAD) in the general population is about 2·6–2·9 per 100 000 inhabitants (1, 2), and CAD is one of the most common causes of ischemic stroke in young adults in western countries (3), occurring at a mean age of 44–47 years (2, 4).

Systematic evaluation of CAD heritability has never been undertaken. Nevertheless, there is reasonable evidence suggesting a genetic predisposition to CAD. Firstly, CAD is associated with rare monogenic connective tissue disorders, such as vascular Ehlers–Danlos syndrome (vEDS) (5) Secondly, there are

several reports of familial cases of CAD in the absence of known connective tissue disorders, representing up to 5% of CAD patients in large hospital-based cohorts (6), although this is certainly an overestimation, due to the recruitment bias in these tertiary referral series. Thirdly, about half of the CAD patients were reported to have connective tissue anomalies revealed by skin biopsy (7–9) that are transmitted according to an autosomal dominant pattern (10, 11). Finally, CAD patients also often present concomitant arterial anomalies, such as fibromuscular dysplasia (12), aortic root dilation (13), hyper-distensibility of the arterial wall (14), or endothelial dysfunction (15). An association with intracranial aneurysms (16), and temporal artery histological changes (17), has been suggested by some authors. It has been hypothesized that CAD patients might 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 (8, 18, 19).

Previous studies investigating possible disease-causing mutations in different candidate genes were mostly negative (20). One CAD family carrying a mutation in the COL3A1 gene, usually responsible for vEDS, was reported, but none of the family members had typical clinical features of vEDS (21). Tentative candidate loci were identified on chromosomes 15q24 and 10q26 in another family with inherited dermal connective tissue alterations (11), but the power of the analysis was insufficient to demonstrate linkage. Other genetic linkage studies, performed in a small number of families, yielded negative results (10, 11, 22, 23). Systematic searching of mutations and linkage studies can be used for CAD patients in whom a Mendelian inheritance is suspected, i.e. either patients with a family history of CAD or patients with associated dermal connective tissue alterations that are inherited in the family. Since familial cases of CAD are relatively rare, and skin biopsies are not feasible on a very large scale, this type of study is restricted to a small subset of CAD patients.

In most cases, CAD is likely to be a multifactorial disease. Case–control genetic association studies are therefore most appropriate in this setting. A few studies have suggested a possible association of CAD with three different genetic variants. The most promising association was found with the C677T polymorphism in the MTHFR gene (24–26) in three different studies, although two are overlapping (24, 25). Another study also found the 677TT genotype to be significantly more frequent in patients with multiple dissections, but the association with all CADs was not significant (27). The positive associations observed with a two base-pair deletion in the 3′UTR region of the COL3A1 gene (22), and the E469K polymorphism in the ICAM-1 gene (28), have not yet been replicated and should therefore be considered with caution. Several other genetic association studies have yielded negative results (27, 29–37). They were all performed on small samples, with <300 subjects per group and in most cases <100.

For other complex genetic diseases, the odds ratios (ORs) associated with individual genetic variants is 1·5 or less; if similar principles apply to CAD, much larger sample sizes will be required to identify such variants (38). Given the relatively low incidence of CAD, only a multicenter genetic association study can provide a sufficiently large sample size to reach adequate statistical power.

Cervical Artery Dissection and Ischemic Stroke Patients (CADISP, http://www.cadisp.org) is a European consortium performing research on CAD and ischemic stroke in young adults, and especially on risk factors, stroke-preventive treatment, and outcome predictors. The present collaborative project consists of a multicenter genetic association study, whose primary aim is to identify genetic polymorphisms associated with an increased risk of CAD. The secondary aim is to identify interactions of genetic variants with several environmental and comorbidity factors.

Patients and methods

Population

The study population consists of three groups: (i) patients with CAD (CAD group), (ii) healthy controls (HC group), and (iii) ischemic stroke patients without CAD (IS group). Only individuals of European ancestry [to avoid stratification bias (39)], aged ≥ 18 years by the time of enrolment, are included. The specific inclusion and exclusion criteria for each group are displayed in Table 1. Patients in the CAD group are consecutive CAD patients with or without associated cerebral ischemia, hospitalized in one of the participating neurological centers (Fig. 1). Patients in the IS group are selected among consecutive patients hospitalized in the same centers for an ischemic stroke without CAD, frequency-matched on age (by 5-year intervals) and gender with the CAD group. Patients are recruited both prospectively and retrospectively for the CAD- and IS groups. Retrospective patients are selected from consecutive lists of patients hospitalized for the qualifying event before the beginning of the study in each center. A trained stroke physician reviews the clinical charts and imaging data of these consecutive patients to identify those fulfilling the CADISP inclusion criteria**.** All eligible retrospective patients hospitalized during a given period of time are invited to take part in the study. For the HC group, DNA of healthy individuals from existing DNA-databases (MONICA-Lille, MONICA-Strasbourg, KORA-Augsburg, and VOBARNO cohorts) will be used as controls for the Belgian, French, German, Italian, and Swiss centers. The other centers are recruiting their own age- and sex-matched healthy controls.

Individuals from the three groups (CAD, IS, and HC) are strictly matched on geographical origin in order to avoid stratification bias (39). The planned sample size is 1100 CAD patients, and 2000 subjects for the HC group. We also plan to include approximately 1100 patients in the IS group. As of October 2008, we have included 1061 CAD patients, 743 IS patients, and 2045 healthy controls. Recruitment is advancing as planned and is expected to be completed by early 2009.

Table 1 Specific inclusion and exclusion criteria for the three groups

	CAD group	IS group	Healthy controls
Inclusion criteria	Typical radiological aspect of dissection* in a cervical artery (carotid, vertebral)	Recent ischemic stroke No sign of CAD on ultrasound and angiography (MR or CT or conventional), performed <7 days after the stroke	Individuals from the general population without a history of vascular disease (MI, stroke, PAD)
Exclusion criteria	Purely intracranial dissection Iatrogenic dissection after an endovascular procedure Monogenic disorder known to cause CAD (e.g. vascular Ehlers–Danlos syndrome)	Possible IS with normal cerebral imaging CAD cannot be ruled out (e.g. persistent arterial occlusion without mural hematoma) Endovascular or surgical procedure on coronary, cervical, or cerebral arteries <48 h Cardiopathies with a very high embolic risk† Arterial vasospasm after subarachnoid hemorrhage Auto-immune disease possibly explaining IS Monogenic disease explaining IS‡	

*Mural hematoma, pseudoaneurysm, long tapering stenosis, intimal flap, double lumen, or occlusion >2 cm above the carotid bifurcation revealing a pseudoaneurysm or a long tapering stenosis after recanalization. †Mechanical prosthetic valves, mitral stenosis with atrial fibrillation, intracardiac tumor, infectious endocarditis, myocardial infarction <4 months. ‡For example, CADASIL, Fabry disease, MELAS, homocystinuria, and sickle cell disease. CAD, cervical artery dissection; IS, ischemic stroke; MI, myocardial infarction; PAD, peripheral artery disease.

Fig. 1 CADISP-recruiting centers. The circles outline geographically close centers that will be analyzed together.

Study protocol

The study protocol was approved by relevant local authorities in all participating centers and is being conducted according to the national rules concerning ethics committee approval and informed consents. During an interview with the participant, a venous blood sample is collected, and a detailed standardized questionnaire is filled in by a stroke physician.

Table 2 Power calculations

Minor allele frequency	*P*-value threshold	Power to detect association (%)	
		OR = 1·5	OR = 1·3
>10%	0·01*	98·0	72·3
	0·001†	90·9	45·1
	5×10^{-7}‡	34·3	3·1
>20%	0·01	99·8	94·2
	0·001	98·6	80·4
	5×10^{-7}	67·9	19·0
>30%	0·01	99·4	98·2
	0·001	99·0	91·6
	5×10^{-7}	72·4	36·0

*Threshold if five independent candidate genetic variants are tested. †Threshold if 50 independent candidate genetic variants are tested. ‡Genome-wide significance (53); these estimations were computed assuming an additive model, and complete LD between the high-risk allele frequency and the marker allele frequency. LD, linkage disequilibrium; OR, odds ratio.

The questionnaire includes family history, past medical history, vascular and other suggested risk factors, clinical symptoms and signs at admission, main results of imaging studies, a standardized connective tissue examination, and information on therapy and outcome at 3 months. Extraction and storing of DNA are conducted according to standard methods.

Analysis strategy

We will perform a genome-wide association study (GWAS) that consists in genotyping very large numbers of single nucleotide polymorphisms (SNPs), 100 000 to 1 million, and for the most recent arrays also copy number variants, distributed across the chromosomes, without any *a priori* hypothesis regarding the underlying pathophysiology. This makes it possible to identify completely novel associations. By taking advantage of linkage disequilibrium [(LD), the correlation among SNPs], current arrays can capture about 80–90% of common variation in HapMap at an $r^2 \geq 0{\cdot}50$ (r^2 being a measure of LD) (40). Indeed, using imputation methods, which rely on information from sets of densely genotyped individuals to infer missing genotypes at untyped variants (41, 42), the association analysis can be extended to all common SNPs described in HapMap (CEU population). The genome-wide association analysis will be performed at the Centre National de Genotypage (http://www.cng.fr/), using the most appropriate high-throughput technology available at the end of the recruitment period.

In addition to the GWAS, which is the most original and promising approach, we also plan to select candidate genes, based on what is known about the pathophysiology of CAD, i.e. mainly among genes involved in connective tissue stability, endothelial function, and inflammatory pathways. Examples of potential candidate genes are provided in supporting information Table S1. On each candidate gene, the SNPs to be studied will be selected based on previous publications, LD, comparative genomics, functional studies, bioinformatics (polymorphism databases and related tools), and possibly also results of the GWAS.

Statistical approach

In a first analysis step, we will compare the genotype frequencies between patients with CAD (CAD group) and healthy controls (HC group). In a second step, in order to assess the specificity of the above associations, we will compare the frequency of the genetic variants selected in the first step between the CAD group and the IS group. This is to determine whether the genetic variants are associated with CAD itself, or are risk factors for stroke in young patients in general.

This study is designed to allow sufficient power to perform both a candidate gene and a genome wide association study. For the comparison between the CAD group (n = 1100) and the HC group (n = 2000), assuming an additive model, we performed power estimations over a range of ORs, high-risk allele frequencies, and significance thresholds, as shown in Table 2 (using the genetic power calculator: http://pngu.mgh.harvard.edu/ ~ purcell/gpc/).

All the analyses will be stratified by geographical region (Fig. 1) to reduce the risk of false-positive associations due to population stratification. In addition, we will also screen each population for latent population substructure (including cryptic relatedness) using suitable programs (43, 44), and correct for this substructure when appropriate.

To confirm our findings, we have planned to test whether the polymorphisms that we will have found to be associated with CAD in the present study are also associated with the same phenotype in other independent populations, to exclude spurious associations. Several additional centers have already expressed an interest to participate in a replication study.

For polymorphisms with definitive evidence of association with CAD, we subsequently plan to genotype additional adjacent polymorphisms, including in particular compelling functional candidates such as nonsynonymous coding SNPs, or SNPs in regulatory regions at transcription factor-binding sites, using bioinformatics tools that provide functional annotation of the human genome. When appropriate, a haplotype analysis will be performed.

For the variants with the strongest evidence of association, we will also search for interactions with several environmental and comorbidity factors such as infection or trauma in the weeks before the dissection, history of migraine, vascular risk factors, or CAD characteristics (e.g. multiple vs. unique, vertebral vs. carotid, with vs. without IS).

Quality control

Each recruiting country provides a quality control of the clinical database, with an independent verification of

randomly selected original case records in each center, to ensure that the data collection has been accurate, which includes a strict verification of the accuracy of the diagnosis for the qualifying event. In addition, quality control filters will be applied after the genotyping, such as exclusion of individuals with a high rate of missing data, with extreme heterozygosity or with non-European ancestry.

Discussion

Studies searching for genetic risk factors of stroke in general were mostly inconclusive (45). The main reasons for this are probably the small sample sizes and the heterogeneity of the stroke phenotype. It is now recommended that genetic studies should use large samples and focus on homogeneous subtypes of stroke (38, 45, 46). Elucidation of the genetic background and potential risk factors of CAD is important, as CAD is a major ischemic stroke subtype in young adults.

CAD is a particularly interesting stroke subtype for several reasons. Firstly, it occurs in young individuals, and previous studies suggest a stronger genetic component in stroke patients younger than 70 years (47–49). Secondly, there is some evidence for a genetic predisposition to CAD, which may be more important than genetic predisposition to stroke in general. Although some results have generated interesting hypotheses, previous genetic association studies on CAD have been mostly disappointing (22, 27, 29–35). The relatively low prevalence of CAD has made it difficult to reach sufficient sample sizes.

The CADISP study is, to our knowledge, the largest genetic association study on CAD, made possible through a multicenter recruitment. Other strengths of this study are: (i) careful phenotyping using stringent inclusion and exclusion criteria based on highly specific radiological characteristics, (ii) use of a standardized questionnaire that includes numerous details on the clinical variables and imaging characteristics, and (iii) analysis strategy using both a genome-wide and a candidate gene approach.

By genotyping large numbers of SNPs distributed across the chromosomes, without requiring an *a priori* hypothesis, GWAS are ideally suited for the discovery of previously unimagined pathways for a given disease. A large number of genome-wide association analyses (http://www.genome.gov/gwastudies/) have recently identified novel genes that increase the risk of developing several complex diseases such as diabetes (50), coronary heart disease (51), and obesity (52). This agnostic approach has proven considerably more successful than the candidate gene approach, which has produced only a very limited number of true, replicable associations over the past decade (38). Although there still remain uncertainties and challenges with respect to several aspects of the genome-wide approach, it has already substantially improved our understanding of many complex traits. This approach may be equally well suited to CAD if sufficiently large populations can be collected.

Although the genome coverage of newer generation arrays is very high (40), it is not complete, and it can therefore be an interesting complement to use a candidate gene approach with dense genotyping of polymorphisms in highly plausible candidate genes, as has recently been proposed by large consortia such as the Candidate gene Association Resource (http://public.nhlbi.nih.gov/GeneticsGenomics/home/care.aspx).

Another originality of the CADISP-study is the inclusion of a second control group consisting of young ischemic stroke patients without CAD, to assess whether the polymorphisms that are found to be more frequent in CAD patients compared with healthy controls are specifically associated with CAD, and not more generally with ischemic stroke in the young.

Limitations are insufficient power to detect rare variants with very small effect sizes, especially using the genome-wide approach (such small associations would, however, have only limited practical implications), and also insufficient power to detect weak gene–environment interactions between rare genetic variants and environmental factors.

The CADISP network will likely provide novel data on genetic susceptibility to CAD. The establishment of this multinational European network including several large stroke centers may hopefully contribute toward an improved understanding of the pathophysiology of CAD and open an avenue for new prevention strategies and treatment options.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1.List of potential candidate genes which could be implicated in the pathogenesis of CAD.

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