Common variation in PHACTR1 is associated with susceptibility to cervical artery dissection.

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Abstract

Cervical artery dissection (CeAD), a mural hematoma in a carotid or vertebral artery, is a major cause of ischemic stroke in young adults although relatively uncommon in the general population (incidence of 2.6/100,000 per year)[1]. Minor cervical traumas, infection, migraine and hypertension are putative risk factors[1–3], and inverse associations with obesity and hypercholesterolemia are described[3,4]. No confirmed genetic susceptibility factors have been identified using candidate gene approaches[5]. We performed genome-wide association studies (GWAS) in 1,393 CeAD cases and 14,416 controls. The rs9349379[G] allele (*PHACTR1*) was associated with lower CeAD risk (odds ratio (OR) = 0.75, 95% confidence interval (CI) = 0.69–0.82; $P = 4.46 \times 10^{-10}$), with confirmation in independent follow-up samples (659 CeAD cases and 2,648 controls; $P = 3.91 \times 10^{-3}$; combined $P = 1.00 \times 10^{-11}$). The rs9349379[G] allele was previously shown to be associated with lower risk of migraine and increased risk of myocardial infarction[6–9]. Deciphering the mechanisms underlying this pleiotropy might provide important information on the biological underpinnings of these disabling conditions.

We organized an international initiative with the aim of collecting clinical data and DNA for the largest possible number of CeAD cases to identify genetic susceptibility loci. We obtained 942 CeAD cases from the Cervical Artery Dissections and Ischemic Stroke Patients (CADISP) study (CADISP-1: 170 Finns and 772 non-Finnish Europeans)[10]. An additional 451 CeAD cases of European origin were recruited specifically for the CADISP-genetics project at European and US centers (CADISP-2). These collections provided a total of 1,393

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Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

AUTHOR CONTRIBUTIONS

Study conception and design were performed by S.D., Y.K., M.L., D.L., P.A. and J.D. Acquisition of data was carried out by S.D., T.M.M., M.K., S.T.E., A. Pezzini, V.T., H.S.M., M.D., R.D., E.T., A.M.S., Y.S., S.A., Y.B., V.C., A.B., A.G., M.S., J.C., C. Lamy, E.M., S.B., L.H.B., A.J.G., P.M., J.J.M., P.S., E.B., B.G., E.G.v.d.H., I.F.-C., K.J., I.W., M.A.N., F.-E.D.L., C.J., Y.-C.C., A.J.M., C. Lichy, L.D., L.K., M.N., P.A.L., T. Brandt, G.B.B., H.-E.W., C.G., T. Böttcher, M.C., D.A., M.A.I., M.M.B.B., A. Padovani, J.F.M., G.K., A.R., B.B.W., E.-B.R., D.Z., T.T., M.L., D.L., P.A. and J.D. Statistical analysis and interpretation of the data were performed by S.D., Y.K., C.W., Y.-C.C., G.C., M.L., P.A. and J.D. The manuscript was drafted by S.D., Y.K., M.L., P.A. and J.D. Critical revision of the manuscript was performed by S.D., Y.K., T.M.M., S.T.E., C.W., M.L., P.A., J.D., A. Pezzini, V.T., H.S.M., E.T., A.M.S., J.C., J.J.M., P.S., I.F.-C., A.J.M., P.A.L., M.A.I., D.Z., T.T., M.L., D.L., P.A. and J.D. Annotation for expression quantitative trait loci was performed by A.D.J. Funding was obtained by S.D., S.T.E., A. Pezzini, V.T., H.S.M., M.D., S.B., A.J.G., P.M., J.J.M., P.S., B.G., F.-E.D.L., C.J., P.A.L., G.B.B., H.-E.W., M.C., D.A., M.M.B.B., J.F.M., A.R., B.B.W., E.-B.R., D.Z., T.T., M.L., D.L., P.A. and J.D.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

CeAD cases of European ancestry for the GWAS (discovery) phase. All CeAD cases were ascertained through departments of neurology specialized in stroke care (Supplementary Figs. 1–3 and Supplementary Note; ClinicalTrials.gov identifier NCT00657969)[11]. DNA from 14,416 controls was available (287 Finns and 14,129 non-Finnish Europeans). We obtained 659 additional CeAD cases that could not be included in the GWAS because of the timing of their inclusion or the availability of only limited amounts of DNA. These cases were used along with 2,648 controls for follow-up of a small number of GWAS results. Finally, to examine the disease specificity of the genetic associations, we recruited 583 individuals with an ischemic stroke attributable to other causes (non-CeAD ischemic stroke: 162 Finns and 421 non-Finnish Europeans) who had similar age, sex and geographical origin characteristics as the CeAD cases. The clinical characteristics of the research subjects are shown in Table 1.

DNA samples were genotyped on an Illumina Human610-Quad or Human660W-Quad BeadChip. We performed imputation to the non-monomorphic SNPs described in the HapMap 2 and 1000 Genomes Project (August 2010 release) CEU (European-ancestry) panels. CADISP-1 and CADISP-2 data were analyzed separately because genotyping was carried out on different platforms (Supplementary Table 1). Moreover, because Finnish participants had a distinct ancestral origin (Supplementary Fig. 4), CADISP-1 was divided into CADISP-1 non-Finnish and CADISP-1 Finnish studies. We performed a fixed-effects inverse variance–weighted meta-analysis after applying a genomic control correction to each of the three GWAS results (CADISP-1 non-Finnish, CADISP-1 Finnish and CADISP-2; Supplementary Note). The quantile-quantile plot for the CeAD GWAS is shown in Supplementary Figure 5. We observed no overall inflation of P values or evidence for significant population stratification (genomic inflation factor $\lambda = 1.032$).

A Manhattan plot of the meta-analysis association results for genotyped SNPs is shown in Supplementary Figure 6 (and in Supplementary Fig. 7 by substudy). The evidence of association with CeAD reached genome-wide significance for two SNPs at two loci. These were rs9349379[G] in intron 2 of *PHACTR1* on chromosome 6p24.1-p23 (OR = 0.75, 95% CI = 0.69–0.82; $P = 4.46 \times 10^{-10}$) and rs11172113[C] in intron 1 of *LRP1* on chromosome 12q13.3 (OR = 0.78, 95% CI = 0.71–0.85; $P = 4.22 \times 10^{-8}$). Overall, we found 77 SNPs (at 51 loci) with association $P < 1 \times 10^{-4}$ (Supplementary Table 2) and 6 SNPs (at 5 loci) with association $P < 1 \times 10^{-5}$ (Table 2).

We restricted further analyses to loci with association $P < 1 \times 10^{-5}$. Two of these SNPs showed nominally significant heterogeneity in effect according to dissection site ($P = 6.49 \times 10^{-3}$ for rs1466535 (*LRP1*) and $P = 0.038$ for rs6820391 (*LNX1*)), with stronger associations for carotid than vertebral dissection (Table 3). None of these markers were associated with non-CeAD ischemic stroke (Supplementary Table 3).

We also identified two loci that were not included in the GWAS genotyping panel but reached genome-wide significance when imputed: rs2163474 in *CCDC102B* on chromosome 18q22.1 (OR = 1.78, 95% CI = 1.50–2.11; $P = 3.86 \times 10^{-11}$) and rs9915775 on chromosome 17q21.1 (OR = 3.39, 95% CI = 2.21–5.19; $P = 1.97 \times 10^{-8}$). Neither showed significant heterogeneity in effect by dissection site ($P = 0.72$ and 0.24, respectively), nor

were they associated with non-CeAD ischemic stroke (Supplementary Table 3). We removed rs9915775 from further consideration because of its low estimated minor allele frequency (MAF = 0.01) and relatively poor imputation quality (R^2 = 0.59).

We examined 6 SNP markers at the 5 most significantly associated loci from the genotyped panel ($P < 1 \times 10^{-5}$ in the GWAS) in the 659 CeAD case and 2,648 control samples available for follow-up (Supplementary Table 4). In addition, we selected two SNPs from the imputed locus at 18q22.1 for genotyping in follow-up samples (Supplementary Table 5). We also genotyped two proxies for two of the most significantly associated SNPs from the GWAS meta-analysis (rs12215208, R^2 = 0.39 with rs9349379; rs6761601, R^2 = 0.67 with rs6741522).

First, we calculated association statistics for the follow-up samples, using a threshold of $P = 5.00 \times 10^{-3}$ to assign significance (Bonferroni correction for ten SNPs; a conservative threshold because of the existence of positive linkage disequilibrium (LD) between some markers); irrespective of significance, we calculated combined association test statistics using meta-analysis and evaluated evidence of heterogeneity between the GWAS and follow-up data (Table 3 and Supplementary Table 5). Second, we applied a Bayesian approach to evaluate the significance of the associations using Wakefield's approximate Bayes factor (Online Methods)[12]. Asymptotic Bayes factor (ABF) and Bayesian false discovery probability (BFDP) values are provided in Supplementary Tables 6 and 7.

rs9349379 (in *PHACTR1*), which provided the strongest evidence of association in the GWAS data, showed significant association with CeAD in the follow-up sample using the Bonferroni-corrected *P*-value threshold described above ($P = 5.00 \times 10^{-3}$), and the result from the meta-analysis of the discovery and follow-up studies maintained the finding of genome-wide significance (OR = 0.77, 95% CI = 0.72–0.83; $P = 1.00 \times 10^{-11}$) (Fig. 1 and Supplementary Fig. 8). Application of the Wakefield Bayesian approach yielded an ABF of 1.70×10^{-10}. Another SNP in *PHACTR1*, rs12215208, selected as a proxy ($P = 2.52 \times 10^{-5}$ in the GWAS), exhibited a nominally significant association in the follow-up samples ($P = 0.015$), but the combined GWAS and follow-up data did not show genome-wide significant association (Supplementary Table 5). In contrast, we found no evidence of association in follow-up samples at rs11172113 in *LRP1*, the second genotyped marker that had genome-wide significant association in the GWAS (Supplementary Fig. 8).

Of the other markers that we examined, one met the criteria for significance in the follow-up samples: rs6820391 in *LNX1* on chromosome 4q12 gave $P = 9.28 \times 10^{-4}$ in the follow-up samples and reached genome-wide significance in the combined meta-analysis (OR = 1.24, 95% CI = 1.15–1.34; $P = 2.36 \times 10^{-8}$) (Supplementary Fig. 8). However, as the Wakefield Bayesian approach yielded an ABF of 3.25×10^{-6}, we consider this association to be only suggestive, requiring further studies for confirmation. Data for the imputed 18q22.1 locus provided no evidence in favor of association in the follow-up collection ($P > 0.15$; Supplementary Table 5).

In sensitivity analyses, stratifying on sex, migraine status and recent cervical trauma status did not modify the genetic associations with CeAD for the top loci (Supplementary Tables

8–10). We observed a nominally significant association of the rs9349379 (*PHACTR1*) risk allele for CeAD with a younger age of onset (Supplementary Table 11). The effect of rs9349379 was more marked in individuals with CeAD without ischemia, whereas the effect of rs6820391 (*LNX1*) was more marked in individuals with CeAD with ischemia, although the confidence intervals largely overlapped and *P* values were similar (Supplementary Table 12). Secondary analyses of association performed separately for carotid and vertebral artery dissections did not yield any genome-wide significant association (Supplementary Table 13).

We did not find any association with CeAD for (i) SNPs reported to be associated with CeAD in candidate gene association studies[13–15]; (ii) SNPs within a 100-kb window from the start and end of *COL3A1*, the gene harboring causal mutations for vascular Ehlers-Danlos syndrome, a rare etiology of CeAD[16]; and (iii) published genome-wide susceptibility SNPs for intracranial aneurysms[17–19] and for thoracic aortic aneurysms and dissection[20] (Supplementary Tables 14–17).

Our most significant association with CeAD was for rs9349379 (*PHACTR1*), which has been associated with myocardial infarction and coronary calcifications in various ancestry groups[6,7,21–26], with effects in the opposite direction of that in CeAD. Two other susceptibility loci for myocardial infarction showed significant associations with CeAD in our data set with effects in the opposite direction of those for CeAD (rs2023938 (*HDAC9*)[7] and rs9982601 (*SLC5A3*, *MRPS6* and *KCNE2*)[6]), and one susceptibility locus for myocardial infarction at 9p21 was associated with CeAD with the same direction of effect (rs3217992 (*CDKN2B-AS1*)[7]) (Supplementary Table 18). No significant association was found between rs9349379 and non-CeAD ischemic stroke in our data set, although such an association was recently described in a larger sample for the ischemic stroke subtype secondary to large artery atherosclerosis (LAA-IS), where the effect was in the opposite direction to the one we observed for CeAD[27]. Another variant predisposing to LAA-IS (rs11984041 (*HDAC9*)[28,29]; $R^2 = 1$ with rs2023938) also displayed an inverse association with CeAD (Supplementary Table 19). Opposite effects of the same genetic variant on different diseases have been described elsewhere[30] and suggest either that the same region harbors different causal variants or that the same causal variant has biological effects with opposite implications for each disease. The vascular risk factor profile[3,4], young age of occurrence[1] and heterogeneous echostructure of carotid arteries in individuals with CeAD[31] all suggest that atherosclerosis is not a predisposing condition for CeAD, in contrast with aortic dissection[32]. With aging and arteriosclerosis, the increased synthesis and reduced degradation of extracellular matrix components, as well as increasing collagen and elastin cross-links, could be hypothesized to make the arterial walls of cervical arteries more resistant to tears[33,34], as could arterial wall calcifications, thus rendering the artery more stiff and resistant to the shear forces of lateral rotation and hyperextension that contribute to CeAD[35].

Migraine is more common in individuals with CeAD than in the community[1,2], and vascular mechanisms are thought to have a key role in the pathophysiology of this disease[36]. Recently, rs9349379 (*PHACTR1*) and rs11172113 (*LRP1*) were identified as migraine susceptibility SNPs[8,9,37]. Moreover, one additional migraine risk variant (rs13208321 in *FHL5*)[9] was associated with CeAD at $P = 6.80 \times 10^{-4}$. All these SNPs showed effects in the

same direction as those for CeAD (Supplementary Table 20). Interestingly, associations with these shared variants were most significant for migraine without aura[9], the migraine subtype most commonly associated with CeAD[38].

PHACTR1 is in a highly conserved genomic region, suggesting that it has a crucial involvement in biological processes[39], but its function is poorly understood. Experimental studies identified a pivotal role for the PHACTR1 protein in vascular tube formation and actin polymerization, suggesting that it possibly has a role in angiogenic processes[40,41]. Upregulation of *PHACTR1* by transforming growth factor (TGF)-β has been described in breast cancer cell lines[42], potentially pointing to a connection with the TGF-β signaling pathway, which is also implicated in genetic predisposition to migraine[9] and has a key role in Marfan and Loeys-Dietz syndromes, two inherited connective tissue disorders causing aortic dissection[43,44]. *In silico* functional annotation obtained from a wide array of published and unpublished expression quantitative trait locus (eQTL) data sets (Supplementary Table 21) provides some support for a functional effect of CeAD-associated SNPs in the 6p24.1-p23 locus on *PHACTR1* expression in whole blood and cerebellum cells (although the SNPs in the databases are in relatively weak LD with rs9349379)[45,46].

Although the follow-up analysis did not provide additional support for association with markers in *LRP1*, this locus remains of interest because of its association with migraine and abdominal aortic aneurysm, with effects in the same direction as in CeAD (Supplementary Table 17)[47].

CeAD is relatively uncommon (~2.6/100,000 cases per year)[48], and the current study is by far the largest genetic study of CeAD thus far (2,052 CeAD cases). Nevertheless, we might have lacked power to detect and replicate some associations, especially for loci showing heterogeneity in effect according to dissection site (Supplementary Table 22). We deliberately chose to focus on the discovery of genetic variants with overarching effects on CeAD risk, as we were underpowered to identify genetic variants underlying carotid or vertebral dissection exclusively. Future studies on larger samples are warranted to explore specific genetic susceptibility factors for carotid and vertebral artery dissections and to determine whether the 12q13.3 (*LRP1*), 4q12 (*LNX1*) and 18q22.1 (*CCDC102B*) loci are associated with CeAD.

In summary, we identified one previously unreported genome-wide significant risk locus for CeAD at *PHACTR1* and additional highly suggestive loci requiring confirmation in future independent studies. *PHACTR1* is also a major susceptibility locus for myocardial infarction and migraine. Understanding the mechanisms by which this locus appears to influence key vascular functions could have major applications for the treatment of these severe and disabling conditions.

URLs

CADISP, http://www.cadisp.com/; International Stroke Genetics Consortium, http://www.strokegenetics.org/.

METHODS

Methods and any associated references are available in the online version of the paper.

ONLINE METHODS

Study population

CeAD cases—We included 942 CeAD cases in the CADISP study in 2004–2009 (CADISP-1: 170 Finns and 772 non-Finnish Europeans)[10]. An additional 451 CeAD cases of European origin were recruited in 2008–2010, exclusively for the CADISP-genetics project, in some CADISP centers and additional European and US centers (CADISP-2). In total, 1,393 CeAD cases of European ancestry were available. All CeAD cases were ascertained through departments of neurology specialized in stroke care. Inclusion criteria, recruiting centers and participant selection are described in Supplementary Figures 1–3 and the Supplementary Note[11].

Individuals with non-CeAD ischemic stroke—As most individuals with CeAD sustained a cerebral ischemia, we planned to test whether genetic variants associated with CeAD were specific for CeAD, possibly through a predisposing vasculopathy, and did not confer generalized susceptibility to cerebral ischemia in young adults that could also predispose to other subtypes of ischemic stroke. We recruited 583 individuals with an ischemic stroke attributable to other causes (non-CeAD ischemic stroke: 162 Finns and 421 non-Finnish Europeans), frequency matched on the basis of age, sex and geographical origin to the CADISP-1 CeAD cases (Supplementary Fig. 3 and Supplementary Note).

Control populations—DNA for 14,416 controls was available. Most controls (n = 14,203: 74 Finns and 14,129 non-Finnish Europeans) were selected from an anonymized control genotype database at the CNG (Centre National de Génotypage) to match cases for ancestry, on the basis of the distribution of eigenvectors (Supplementary Fig. 4 and Supplementary Note)[50]. In addition, 213 Finnish controls were recruited (Supplementary Note). Although controls were not screened for CeAD, given the low disease incidence in the community, a misclassification bias is unlikely.

Genotyping, quality control filters and imputation

DNA samples were genotyped on an Illumina Human610-Quad or Human660W-Quad BeadChip (Supplementary Table 1 and Supplementary Note). After quality control (Supplementary Note), we used 472,862 autosomal SNPs and 10,029 X-chromosomal SNPs for analyses. We performed genotype imputation to the non-monomorphic SNPs described in the HapMap 2 and 1000 Genomes Project (August 2010 release) CEU panels (Supplementary Note). Only SNPs with an imputation score of $R^2 > 0.3$ and a MAF of >0.01 were used for analysis.

Follow-up studies

We sought to replicate our strongest association signals in an independent sample comprising 659 CeAD cases, mostly drawn from existing databases of ischemic stroke

(recruited through neurology departments specialized in stroke care according to the same inclusion criteria as in the discovery GWAS), and 2,648 controls, all of European ancestry (Supplementary Table 4). Some of these (85 CeAD cases and 998 controls) had already been genotyped elsewhere on a genome-wide chip (Supplementary Table 1 and Supplementary Note). In addition, DNA samples from 238 CeAD cases were genotyped across the genome, and 1,584 controls were genotyped on a custom chip including the SNPs yielding the most significant associations in the GWAS (Supplementary Table 1 and Supplementary Note). Finally, DNA samples from 391 additional CeAD cases and 162 controls, recruited in the same centers as other individuals included in the discovery or follow-up analyses, were genotyped for the top 5 genotyped loci and the top imputed locus (1–2 SNPs per locus) using KASPAR technology. Of these 714 follow-up CeAD cases, 55 were excluded because of the unavailability of information on the dissection site, leaving us with 659 CeAD cases for analysis. To avoid population stratification bias, the association of the top SNPs with CeAD was analyzed separately by inclusion region (see Supplementary Table 4 for the matching algorithm), and meta-analysis (fixed effects, inverse variance weighted) was performed on the results.

Statistics

Genome-wide association analysis comparing CeAD cases to healthy controls—Analyses were based on an additive genetic model. We used logistic regression to estimate OR values with corresponding 95% CIs, adjusting for sex. The CADISP-1 and CADISP-2 cohorts were analyzed separately because genotyping was carried out on different platforms (Supplementary Table 1). Moreover, because Finnish participants had a distinct ancestral origin relative to other populations (Supplementary Fig. 4), CADISP-1 was divided into CADISP-1 non-Finnish and CADISP-1 Finnish cohorts. Hence, sample sizes (CeAD-cases/controls) were as follows: 772/8,972 for CADISP-1 non-Finnish, 170/287 for CADISP-1 Finnish and 451/5,157 for CADISP-2. The first ten principal component scores were used as covariates for the CADISP-1 non-Finnish and CADISP-2 cohorts, and the first principal component score was used for the CADISP-1 Finnish cohort (Supplementary Note). Genomic control was applied to each of the three GWAS results (CADISP-1 non-Finnish, CADISP-1 Finnish and CADISP-2). Thereafter, a combined analysis was performed by inverse variance–weighted meta-analysis with a fixed-effects model (Supplementary Note). $P < 5 \times 10^{-8}$ was considered genome-wide significant.

Follow-up study—Because of the limits on DNA availability, six loci (ten SNPs) were chosen for follow-up genotyping in the phase 3 samples uniquely on the basis of statistical rankings in the GWAS meta-analysis of phase 1 and 2 samples (CADISP-1 and CADISP-2). We examined 6 SNP markers at the 5 most significantly associated loci from the genotyped panel ($P < 1 \times 10^{-5}$ in the GWAS) in the 659 CeAD and 2,648 control samples available for follow-up. In addition, two SNPs from the imputed locus on chromosome 18q22.1 were selected for genotyping in follow-up samples (Supplementary Table 5). We also genotyped two proxies for two of the most significantly associated SNPs ($P < 1 \times 10^{-5}$) from the GWAS meta-analysis (rs12215208, $R^2 = 0.39$ with rs9349379; rs6761601, $R^2 = 0.67$ with rs6741522).

First, we calculated the association statistics for the follow-up samples, using a threshold of $P = 5.00 \times 10^{-3}$ to assign significance (with Bonferroni correction for ten SNPs, which is conservative because of positive LD between some markers); irrespective of significance, we calculated combined association test statistics using meta-analysis and evaluated evidence of heterogeneity between GWAS and follow-up data. Second, we applied a Bayesian approach to evaluate the significance of associations using Wakefield's approximate Bayes factor[12]. ABF and BFDP values are provided in Supplementary Tables 6 and 7.

Bayesian approach—We followed the approach developed by Wakefield[12] to consider the posterior odds on the null hypothesis (no association of a SNP with CeAD) for sequential studies from a Bayesian perspective. In summary, we obtained estimates of the log relative risk $\hat{\theta}$, standard error $\sqrt{V}$ and Wald statistic $z^2 = \hat{\theta}^2 / V$ for a SNP from the logistic regression model (based on an additive genetic model), which was performed as our primary analysis to generate frequentist *P* values (Table 3 and Supplementary Table 5). Thereafter, we calculated Wakefield's ABF as follows:

$$\mathrm{ABF} = \sqrt{\frac{W}{RV_1V_2}} \exp\left\{-\frac{1}{2}\left(z_1^2 RV_2 + 2z_1 z_2 R\sqrt{V_1V_2} + z_2^2 RV_1\right)\right\}$$

where W is the variance on the prior for the effect size, $R = W/(V_1W + V_2W + V_1V_2)$, V_1 and V_2 are squared standard errors, and z_1 and z_2 are Wald statistics, each from the discovery GWAS (stages 1 and 2 combined) and the follow-up study (stage 3). A smaller ABF value corresponds to more evidence of the alternative hypothesis, i.e., the association of a SNP with CeAD.

Wakefield proposed three different specifications of priors for the log relative risk. Because our aim in this study was to find common susceptibility variants for CeAD, we followed an effect-MAF independence prior as $W = \left\{\log \mathrm{RR}_u / \Phi^{-1}(1-q)\right\}^2$, with $\mathrm{RR}_u = 1.5$. $\mathrm{RR_u}$ is an upper value above which we believe that relative risks will occur with low probability; q is the prior probability of a relative risk above $\mathrm{RR_u}$; Φ (.) is the distribution function of a standard normal random variable.

Considering PO = $\pi_0/(1-\pi_0)$ to be the prior odds on the null hypothesis, where π_0 is the prior probability of the null (Pr[H_0]), the posterior probability of H_0 is given by the BFDP, with BFDP = ABF × PO/(1 + ABF × PO).

Specificity analysis—We tested whether the SNPs yielding the most significant associations with CeAD were also associated with non-CeAD ischemic stroke. We ran a logistic regression adjusted for sex and principal components, as described above, for non-Finnish Europeans (421 cases and 8,972 controls) and Finns (162 cases and 287 controls), followed by a fixed-effects inverse variance–weighted meta-analysis. Statistical significance was defined by false discovery rate (FDR)-corrected $P < 0.05$.

Sensitivity analyses—For the most significant associations, we stratified on and tested for interaction with sex, and we examined whether the most significant SNPs predicted an earlier age of onset of CeAD. We then evaluated the stability of the associations according to dissection site (carotid or vertebral) and the presence of cerebral ischemia (ischemic stroke or transient ischemic attack). Finally, we performed analyses stratified on the presence of two CeAD risk factors, migraine and cervical trauma in cases; case-only association analyses of the top CeAD SNPs with dissection site, the presence of cerebral ischemia, migraine and trauma were used as surrogate tests of heterogeneity or interaction, with $P < 0.05$ being considered significant[51].

Candidate gene approach—We examined the associations of CeAD with published CeAD susceptibility SNPs and with SNPs in *COL3A1*, the gene harboring causal mutations for vascular Ehlers-Danlos syndrome, a rare etiology of CeAD[16]. We also tested for pleiotropic effects of susceptibility SNPs for intracranial aneurysms, thoracic aortic aneurysms and dissections, other subtypes of ischemic stroke and migraine (Supplementary Note). An FDR-corrected *P* value of <0.05 was considered to be statistically significant. We performed a sign test to evaluate whether associations of CeAD with alleles previously associated with migraine and ischemic stroke subtypes involved the same risk alleles more often than predicted by chance. A right-tailed *P* value of 0.05 was considered to be statistically significant.

Ethics

The study protocol was approved by local authorities in all participating centers and conducted according to national rules concerning ethics committee approval and informed consent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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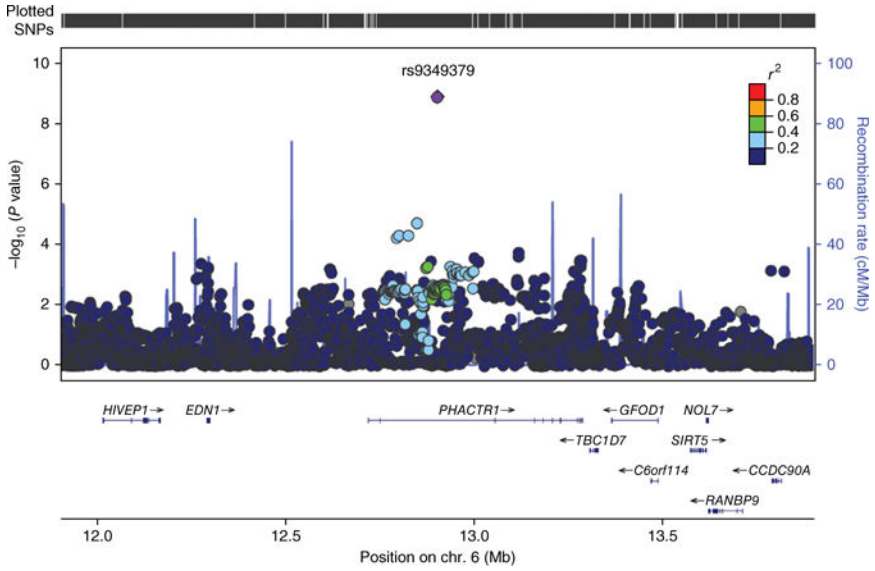

Figure 1.

Regional association plot centered on rs9349379 (*PHACTR1*). Regional plot for associations in the region centered on rs9349379, drawn using LocusZoom software[49]. All SNPs, on the basis of 1000 Genomes Project imputed results (dots), are plotted with their GWAS meta-analysis association *P* values against their genomic position. The color of the dots represents the LD between SNPs. The purple line represents the estimated recombination rates. Genes and exons are shown as dark blue arrows and vertical lines, respectively.

Table 1

Baseline clinical characteristics of the study subjects

		CeAD cases							
		GWAS							
		CADISP-1		CADISP-2		Follow-up studies		Non-CeAD ischemic stroke	
n		942				659		583	
Mean age (s.d.)		44.2	(10.0)	44.6	(10.5)	43.6	(9.4)	44.6	(10.5)
Men, *n* (%)		544	(57.7)	264	(58.5)	387	(58.7)	257	(61.2)
Location	Carotid[a], *n* (%)	603	(64.0)	253	(60.7)[b]	375	(56.9)	–	
	Vertebral, *n* (%)	305	(32.4)	147	(35.3)[b]	256	(38.8)	–	
	Both, *n* (%)	33	(3.5)	17	(4.0)[b]	26	(3.9)	–	
Clinical presentation	Cerebral ischemia[c], *n* (%)	729	(77.4)	333	(79.5)[b]	544	(82.5)	–	
	Ischemic stroke, *n* (%)	606	(64.3)	281	(67.1)[b]	508	(77.1)	–	

CeAD, cervical artery dissection.

[a] Internal carotid artery dissection (one additional case had a common carotid artery dissection in CADISP-1).

[b] Information on dissection site and associated ischemia was missing for 34 and 32 cases, respectively, in CADISP-2.

[c] Cerebral ischemia corresponds to ischemic stroke or transient ischemic attack (including transient monocular blindness).

Table 2

SNPs yielding the most significant associations with CeAD in the GWAS

SNP[a]	Chr.	Position (bp)	EA	Nearest gene[b]	Stage	EAF	OR (95% CI)	*P*
rs12402265	1	59,463,190	A	*FGGY*	CADISP-1 non-Finnish	0.26	1.21 (1.07–1.36)	2.70×10^{-3}
					CADISP-1 Finnish	0.28	1.16 (0.87–1.54)	0.315
					CADISP-2	0.28	1.28 (1.10–1.49)	1.30×10^{-3}
					Meta-analysis		1.23 (1.13–1.35)	6.12×10^{-6}
rs6741522	2	185,544,143	A	*ZNF804A*	CADISP-1 non-Finnish	0.14	1.33 (1.15–1.54)	1.99×10^{-4}
					CADISP-1 Finnish	0.10	1.32 (0.85–2.05)	0.230
					CADISP-2	0.13	1.38 (1.14–1.66)	1.21×10^{-3}
					Meta-analysis		1.34 (1.19–1.50)	5.65×10^{-7}
rs6820391	4	54,109,453	A	*LNX1*	CADISP-1 non-Finnish	0.29	1.21 (1.07–1.36)	2.33×10^{-3}
					CADISP-1 Finnish	0.29	1.15 (0.86–1.54)	0.345
					CADISP-2	0.28	1.26 (1.09–1.47)	2.69×10^{-3}
					Meta-analysis		1.23 (1.12–1.35)	6.35×10^{-6}
rs9349379	6	13,011,943	G	*PHACTR1*	CADISP-1 non-Finnish	0.40	0.76 (0.68–0.86)	8.90×10^{-6}
					CADISP-1 Finnish	0.45	0.84 (0.63–1.10)	0.216
					CADISP-2	0.40	0.73 (0.62–0.84)	3.39×10^{-5}
					Meta-analysis		0.75 (0.69–0.82)	4.46×10^{-10}
rs11172113	12	55,813,550	C	*LRP1*	CADISP-1 non-Finnish	0.38	0.77 (0.68–0.86)	1.66×10^{-5}
					CADISP-1 Finnish	0.39	0.90 (0.69–1.18)	0.456
					CADISP-2	0.40	0.77 (0.67–0.89)	7.61×10^{-4}
					Meta-analysis		0.78 (0.71–0.85)	4.22×10^{-8}
rs1466535[c]	12	55,820,737	A	*LRP1*	CADISP-1 non-Finnish	0.32	0.75 (0.66–0.85)	1.06×10^{-5}
					CADISP-1 Finnish	0.28	1.11 (0.83–1.49)	0.473
					CADISP-2	0.34	0.80 (0.68–0.93)	4.04×10^{-3}
					Meta-analysis		0.80 (0.73–0.88)	2.07×10^{-6}

Only results for genotyped SNPs with $P < 1 \times 10^{-5}$ are presented here (for all associations with $P < 1 \times 10^{-4}$, see Supplementary Table 2). Chr., chromosome; EA, effect allele (minor allele); EAF, effect allele frequency in controls. Alleles and chromosomal positions were identified on the basis of the plus strand of the NCBI Build 36 reference genome.

[a] rs9349379, rs11172113, rs1466535 and rs6820391 are intronic; rs6741522 and rs12402265 are intergenic.

[b] The names for the nearest genes are shown according to the Human Gene Organization (HUGO) Gene Nomenclature System.

[c] rs1466535 is in LD with rs11172113 ($r^2 = 0.72$).

Table 3

SNPs yielding the most significant associations with CeAD and their proxies in GWAS and follow-up studies

							GWAS meta-analysis (*n* = 1,393/14,416)		Follow-up meta-analysis (*n* = 659/2,648)		Overall meta-analysis (*n* = 2,052/17,064)		
SNP	**Chr.**	**EA**	**EAF**	**Gene**	**CeAD site**	**P_{het} CeAD site[b]**	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**P_{het}**
rs12402265[a]	1	A	0.27	*FGGY*	All	0.70	1.23 (1.13–1.35)	6.12×10^{-6}	1.21 (1.04–1.40)	0.012	1.23 (1.14–1.33)	2.30×10^{-7}	0.83
					Carotid		1.23 (1.10–1.38)	2.95×10^{-4}	1.40 (1.17–1.68)	2.52×10^{-4}	1.28 (1.16–1.41)	5.46×10^{-7}	0.24
					Vertebral		1.27 (1.10–1.47)	1.36×10^{-3}	1.05 (0.85–1.31)	0.64	1.20 (1.06–1.35)	3.47×10^{-3}	0.16
rs6741522	2	A	0.13	*ZNF804A*	All	0.64	1.34 (1.19–1.50)	5.65×10^{-7}	1.09 (0.91–1.31)	0.36	1.26 (1.15–1.39)	2.29×10^{-6}	0.06
					Carotid		1.37 (1.19–1.57)	1.17×10^{-5}	1.05 (0.83–1.32)	0.68	1.27 (1.13–1.44)	7.33×10^{-5}	0.06
					Vertebral		1.32 (1.10–1.60)	3.53×10^{-3}	1.24 (0.96–1.61)	0.10	1.29 (1.11–1.51)	8.92×10^{-4}	0.69
rs6820391	4	A	0.29	*LNX1*	All	0.038	1.23 (1.12–1.35)	6.35×10^{-6}	1.28 (1.10–1.47)	9.28×10^{-4}	1.24 (1.15–1.34)	2.36×10^{-8}	0.68
					Carotid		1.30 (1.16–1.45)	3.88×10^{-6}	1.29 (1.08–1.55)	4.84×10^{-3}	1.30 (1.18–1.43)	6.41×10^{-8}	0.97
					Vertebral		1.09 (0.94–1.26)	0.28	1.23 (1.00–1.52)	0.049	1.13 (1.00–1.28)	0.042	0.33
rs9349379	6	G	0.40	*PHACTR1*	All	0.52	0.75 (0.69–0.82)	4.46×10^{-10}	0.81 (0.71–0.94)	3.91×10^{-3}	0.77 (0.72–0.83)	1.00×10^{-11}	0.36
					Carotid		0.76 (0.68–0.85)	1.27×10^{-6}	0.76 (0.64–0.90)	1.79×10^{-3}	0.76 (0.69–0.83)	8.24×10^{-9}	0.96
					Vertebral		0.72 (0.62–0.84)	1.38×10^{-5}	0.92 (0.75–1.13)	0.42	0.79 (0.70–0.88)	6.32×10^{-5}	0.06
rs11172113	12	C	0.39	*LRP1*	All	0.061	0.78 (0.71–0.85)	4.22×10^{-8}	0.93 (0.81–1.07)	0.34	0.82 (0.76–0.89)	3.03×10^{-7}	0.03
					Carotid		0.74 (0.66–0.83)	1.14×10^{-7}	0.88 (0.75–1.05)	0.16	0.78 (0.71–0.86)	1.90×10^{-7}	0.08
					Vertebral		0.89 (0.77–1.03)	0.11	0.99 (0.81–1.21)	0.93	0.92 (0.82–1.04)	0.17	0.39
rs1466535[a]	12	A	0.32	*LRP1*	All	6.49×10^{-3}	0.80 (0.73–0.88)	2.07×10^{-6}	0.92 (0.80–1.07)	0.30	0.83 (0.77–0.90)	4.94×10^{-6}	0.10
					Carotid		0.73 (0.65–0.82)	2.50×10^{-7}	0.85 (0.71–1.01)	0.07	0.76 (0.69–0.84)	1.17×10^{-7}	0.18

SNP	Chr.	EA	EAF	Gene	CeAD site	P_{het} CeAD site[b]	GWAS meta-analysis (n = 1,393/14,416) OR (95% CI)	GWAS meta-analysis (n = 1,393/14,416) P	Follow-up meta-analysis (n = 659/2,648) OR (95% CI)	Follow-up meta-analysis (n = 659/2,648) P	Overall meta-analysis (n = 2,052/17,064) OR (95% CI)	Overall meta-analysis (n = 2,052/17,064) P	Overall meta-analysis (n = 2,052/17,064) P_{het}
					Vertebral		0.97 (0.84–1.12)	0.68	0.99 (0.80–1.22)	0.92	0.98 (0.87–1.10)	0.69	0.87

Chr., chromosome; EA, effect allele (minor allele); EAF, effect allele frequency (in controls from the GWAS); OR, odds ratio; CI, confidence interval; P_{het}, P value for heterogeneity between the discovery GWAS and the follow-up sample.

[a] These SNPs were imputed (HapMap 2) in the Maryland follow-up sample with R^2 = 0.93 (rs1466535) and 1 (rs12402265).

[b] The P value of association with the dissection site in a case-only analysis.