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Rare genetic variants in patients with cervical artery dissection

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Abstract

Introduction: The potential role of genetic alterations in cervical artery dissection (CeAD) pathogenesis is poorly understood. We aimed to identify pathogenic genetic variants associated with cervical artery dissection by using whole exome sequencing.

Patients and methods: CeAD-patients with either a family history of cervical artery dissection (f-CeAD) or recurrent cervical artery dissection (r-CeAD) from the CeAD-databases of two experienced stroke centres were analysed by whole exome sequencing.

Variants with allele frequency <0.05 and classified as pathogenic by predicting algorithms (SIFT or Polyphen-2) or the ClinVar database were explored. First, we analysed a panel of 30 candidate genes associated with arterial dissection (any site) or aneurysm according to the OMIM (online Mendelian Inheritance of Men) database. Second, we performed a genome-wide search for pathogenic variants causing other vascular phenotypes possibly related to cervical artery dissection.

Findings were classified as CeAD-causing (pathogenic variants in genes from the arterial dissection or aneurysm panel) or suggestive (pathogenic variants in genes associated with other vascular phenotypes and variants of unknown significance in genes from the arterial dissection or aneurysm panel). All other variants were classified as benign/uncertain.

Results: Among 43 CeAD-patients, 28 patients (17 pedigrees) had f-CeAD and 15 had r-CeAD. No CeAD-causing variants were identified in r-CeAD patients. Among f-CeAD-patients, 5/17 pedigrees carried CeAD-causing variants in COL3A1, COL4A1, COL4A3, COL4A4, COL5A1, COL5A2 and FBN1. Suggestive variants in ABCC6, COL3A1, COL5A2, MEF2A, and RNF213 were detected in three pedigrees with f-CeAD and six patients with r-CeAD.

Discussion and conclusion: CeAD-causing variants were rare and exclusively found in f-CeAD-patients, suggesting differences between the genetic architectures of f-CeAD and r-CeAD. The identified variants indicate a high genetic heterogeneity of the study sample.

Keywords

Cerebrovascular disease/stroke, vascular disease, genetics, cervical artery dissection, whole exome sequencing

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Introduction

Cervical artery dissection (CeAD) – a major cause of stroke in young adults – is characterised by a hematoma within the arterial wall or by a tear in the intimal layer of the internal carotid artery (ICA) or the vertebral artery (VA).[1] The pathogenesis of CeAD is poorly understood. Trauma or other mechanical trigger events seem to precede CeAD in less than half of the patients.[2] Alterations of the structure of the arterial wall or of the connective tissue composition were suggested by the findings of genetic analyses as well as microscopic and autopsy studies.[3–5] Electron microscopic studies revealed minor abnormalities of the morphology of collagen fibrils and elastic fibres in skin biopsy specimens of about 60% of CeAD patients.[3] These findings suggest an underlying generalised connective tissue alteration manifesting as arterial vulnerability.

The assumed existence of constitutional risk factors for CeAD was recently confirmed by genome-wide SNP-microarray studies identifying association with variants in the genes encoding PHACTR1 (phosphatase and actin regulator 1) and LRP1 (LDL receptor related protein 1) and with large-size copy number variants affecting cardiovascular system development.[6,7] Only very few studies have reported genetic alterations in CeAD using next generation sequencing techniques. In a previous whole exome sequencing (WES) study, we identified pathogenic mutations in different genes related to arterial connective tissue phenotypes in seven Caucasian pedigrees with a family history of CeAD (f-CeAD) and two pairs of affected monozygotic twins.[8] This study had a limited focus by including f-CeAD patients only and identification of genetic variants in a panel of only 11 pre-defined candidate genes.

In the present study, we aimed to expand our search for genetic variants associated with CeAD. We therefore performed WES in patients with either a familial history of CeAD (f-CeAD) or a recurrent CeAD $\geq$3 month after the first CeAD (r-CeAD). This selection based on the assumption that patients with f-CeAD or r-CeAD were more likely to carry disease-causing mutations than sporadic patients with single CeAD events. In a two-step approach, we first performed a targeted analysis of 30 genes associated with arterial dissection or aneurysm which was then followed by a genome-wide exploration of pathogenic variants causing other vascular phenotypes.

Patients and methods

Patient selection and variables

Based on the CeAD databases of the departments of Neurology and Stroke Centers of the University Hospital Basel, Switzerland, and the University Hospital Heidelberg, Germany, we selected all patients who fulfilled the following criteria: patients (1) first CeAD between 2004 and 2017; (2) absence of a known connective tissue disorder; and (3) informed consent to participate in the study; (4) with a family history of CeAD (f-CeAD) or with recurrent CeADs (r-CeAD) applying criteria used in prior research.[8–10] In brief, the latter criteria were defined as follows. F-CeAD was defined by a reported history of CeAD in parents, siblings, children or any other defined relative of the index patient;[9] r-CeAD: CeAD occurring later than one month after the index CeAD which was confirmed by magnetic resonance imaging in a vessel location different from the primarily affected vessel.[10] In all participants, CeAD diagnosis was verified by arterial imaging applying the following, widely accepted diagnostic criteria (presence of at least one): mural hematoma, aneurysmal dilatation, long tapering stenosis, intimal flap, double lumen, or occlusion $\geq$2 cm above the carotid bifurcation revealing an aneurysmal dilatation or a long tapering stenosis after recanalisation in a cervical artery.[11]

The study sample included 43 patients from 32 pedigrees. For the current analysis, the following variables from the CeAD databases were extracted: age (at onset of first CeAD), sex, site of dissection (ICA or VA) and presence of familial or recurrent CeAD.

Exome sequencing analysis and prioritisation

Peripheral blood was used for DNA extraction. Exome sequencing was performed at the German Research Centre for Environmental Health, Helmholtz Zentrum München, on a Genome Analyser IIx system (Illumina) after in-solution enrichment of exonic sequences (SureSelect Human All Exon 38Mb kit, Agilent). Read alignment was performed with Burrows-Wheeler Aligner (BWA, version 0.5.8) to the human genome assembly hg19. Single-nucleotide variants (SNVs) were detected with SAM (Sequence Alignment/Map) tools (v 0.1.7), as described previously.[8]

First, we analysed variants in a panel of 30 candidate genes associated with arterial dissection (any site) or aneurysm according to the OMIM (online Mendelian Inheritance of Men) database (Supplementary Table 1). Pathogenic variants in these genes caused monogenic disorders associated with arterial phenotypes including Ehlers–Danlos syndrome (EDS), Marfan syndrome, Loeys-Dietz syndrome, Alport syndrome, familial thoracic aortic aneurysms and dissections, and arterial tortuosity syndrome. Single nucleotide variants (SNVs) findings were prioritised if they (1) had a coverage (depth) of $\geq$40 reads

and (2) caused nonsense amino acid substitutions in the encoded gene product, or a missense substitution rated as pathogenic by at least one of the following scores including SIFT (*S*orting *I*ntolerant *F*rom *T*olerant)[12] score <0.05, polyphen-2[13] >0.95, or a ClinVar[14] database evaluation as pathogenic/likely pathogenic and (3) were identified in all affected subjects from a pedigree.

In a second step, we performed a genome-wide search for pathogenic/likely pathogenic variants that may possibly play a role in the pathogenesis of CeAD. To do so, we selected all gene variants with allele frequency <0.05 in the in-house database of the Helmholtz Center Munich ($n > 15{,}000$) that were predicted as pathogenic by SIFT, Polyphen-2 or ClinVar (criteria see above) affecting the same gene in at least two different pedigrees.

In Supplementary Table 2, finally, we present all variants that either (1) were pathogenic according to the ClinVar database; or (2) resulted into nonsense-substitutions or (3) induced a frameshift or (4) had both Polyphen-2 score >0.95 and SIFT score <0.05 and (5) occurred in all affected relatives of a pedigree.

Interpretation of exome findings

We categorised our findings based on the following operationalised definitions: Variants found in the 30 genes associated with arterial dissection or aneurysm were considered 'CeAD-causing' (i.e. disease-causing with relation to CeAD) if they were rated as pathogenic in the ClinVar database or had a SIFT score <0.05 as well as a polyphen-2 score >0.95. Variants in the panel of 30 dissection/aneurysm genes with uncertain (or unknown) significance (variants of uncertain significance in ClinVar, or with conflicting prediction of pathogenicity in SIFT and polyphen-2) were considered as 'suggestive', as well as pathogenic variants affecting two pedigrees and associated with other vascular phenotypes. All other observed exome variants were considered as insignificant and remain unreported in the current study.

The Fisher exact test was used to compare the frequency of 'CeAD-causing' variants in the f-CeAD and r-CeAD subgroups

Ethical approval and informed consent

The study protocol was approved by relevant local authorities in both centres and complied with national regulations concerning ethics committee approval and informed consent. All patients gave written informed consent prior to study participation.

Results

The study sample comprised 43 CeAD patients; 28 f-CeAD (17 pedigrees) and 15 r-CeAD patients (Table 1). Two patients with f-CeAD also had recurrent events, resulting in analysis of 17 r-CeAD patients in total. Mean age at onset of CeAD was 37.4 ± 10.0 years for the patients with f-CeAD (20 women, 8 men) and 39.7 ± 8.5 years for the r-CeAD patients (10 women, 5 men). Of four pedigrees with f-CeAD, only a single affected family member was available for analysis. Of two affected identical twin pairs, only DNA of a single patient was analysed. Candidate gene studies of patients from families 1,2,3,6,7,8,10,11,15,16 and 26 were presented in earlier studies.[8,15,16]

The targeted analysis of 30 genes related to aneurysms or dissection yielded CeAD-causing findings in five patients (Table 2). These five patients all had f-CeAD, whereas no CeAD-causing variants were found among the r-CeAD patients (Fisher exact Test; $p = 0.046$). The observed CeAD-causing variants were found in genes associated with different connective tissue syndromes: (1) Marfan syndrome, due to a FBN1 Arg244Trp missense variant; (2) vascular Ehlers–Danlos syndrome due to a COL3A1 Gly214Ser missense variant; (3) HANAC syndrome (hereditary angiopathy with nephropathy, aneurysms, and muscle cramps) due to a COL4A1 Pro116Leu missense variant; (4) digenic Alport syndrome due to missense variants in COL3A3 (Leu1474Pro) and in COL3A4 (Gly972Arg) and (5) classic Ehlers Danlos syndrome due to two different missense mutations in COL5A1 (Arg65Trp and Val 172Phe). Another patient with a family history of CeAD carried two suggestive missense variants affecting TypV collagen (COL5A2 Pro1103Leu and COL5A1 Thr1757Met). Additional suggestive variants were found in COL3A1 and in COL5A2 in two patients with r-CeAD.

The subsequent genome-wide search for variants in genes with pathogenic variants in at least two different pedigrees and associated with other vascular phenotypes yielded the following findings. Suggestive variants in MEF2A (myocyte enhancer factor 2A) were found in two pedigrees with familial CeAD. Further suggestive variants in ABCC6 (ATB binding cassette subfamily C member 6, associated with arterial calcification) and in RNF213 (ring finger protein 213, associated with fibromuscular dysplasia) were identified in patients with recurrent CeAD. A total of 3163 pathogenic gene variants identified in the study sample are shown in Supplementary Table 2.

Discussion

This comprehensive genetic analysis of a selected sample of CeAD patients with either familial or

Table 1. Study sample of patients with f-CeAD or r-CeAD.

Family ID	Patient ID	Sex	Age	First event	Recurrent event
Two affected relatives from f-CeAD family					
1	1	F	38	ICA-bilateral	
1	2	F	32	ICA-le	
2	3	F	41	ICA-le	
2	4	M	43	ICA-le	
3	5	F	19	ICA-ri	
3	6	F	18	ICA-ri	
4	7	F	36	ICA-ri	
4	8	F	37	ICA-le	
5	9	F	49	ICA-le	
5	10	F	31	ICA-ri	
6	11	F	29	ICA-bilateral	
6	12	M	53	ICA-le	
7	13	F	33	ICA-bilateral	
7	14	M	37	VA-ri	
8	15	F	39	ICA-le	
8	16	M	45	ICA-le	
9	17	F	26	VA-le	
9	18	F	55	VA-le	
10	19	M	31	VA-le	
10	20	M	31	ICA-le	VA-ri/ICA-ri/ICA-le
11	21	F	36	ICA-ri	
11	22	F	37	ICA-le	ICA-le
Single patients from f-CeAD family					
12	23	F	59	ICA-le	
13	24	F	49	VA-ri	
14	25	F	30	ICA-bilateral	
15	26	M	42	ICA-ri	
16	27	M	45	ICA-ri	
17	28	F	27	ICA-ri	
Patients with r_ceAD					
18	29	F	34	ICA-le	VA-ri
19	30	F	47	ICA-le	ICA-ri
20	31	M	52	ICA-le	ICA-ri
21	32	F	31	ICA-le	ICA-ri
22	33	F	36	ICA-ri, VA-le	VA-le
23	34	F	41	ICA-ri	VA-bilateral
24	35	F	28	VA-bilateral	VA-ri
25	36	F	41	ICA-le	ICA-ri
26	37	M	34	ICA-le	ICA-ri
27	38	M	54	ICA-ri	ICA-le
28	39	M	50	ICA-le	ICA-ri
29	40	M	41	ICA-le	ICA-ri, VA-ri
30	41	F	29	ICA-ri	VA-ri
31	42	F	32	VA-bilateral	ICA-le
32	43	F	45	ICA-ri	ICA-le

Note: Two affected individuals were analysed from 14 pedigrees with familial CeAD. From the remaining four pedigrees with familial CeAD, only one affected patient was analysed.
ICA: internal carotid artery; VA: vertebral artery; -ri: right side; -le: left side; m: male; f:female;/indicates subsequent events within one individual.

recurrent CeAD had the following key findings: (1) CeAD-causing variants were identified in 5 out of 17 families with f-CeAD, but in none of the patients with r-CeAD; (2) suggestive variants were found in nine further families; (3) Genetic findings were highly heterogeneous.

The findings challenge our assumption that patients with r-CeAD and f-CeAD have a common genetic

Table 2. Rare exome findings in the study sample.

Pedigree	Gene	Description	Variant	dbSNP ID	Consequences	AF	Associated phenotype	Impact
f-CeAD								
2	MEF2A	Myocyte enhancer factor 2A	Pro279Leu	rs121918529	Pathogenic	0.0012	Coronary artery disease, AD, 1	Suggestive
3	COL3A1	Collagen type III α1 chain	Gly324Ser	Unknown	Pathogenic	<0.0001	EDS, type 4	Disease-causing
4	MEF2A	Myocyte enhancer factor 2A	Pro279Leu	rs121918529	Pathogenic	0.0012	Coronary artery disease, AD, 1	Suggestive
5	FBN1	Fibrillin 1	Arg2544Trp	rs369294972	Pathogenic	<0.0001	Marfan syndrome	Disease-causing
6	COL4A1	Collagen type IV α1 chain	Pro116Leu	rs538816765	Pathogenic	<0.0001	HANAC syndrome	Disease-causing
13	COL5A1	Collagen type V α1 chain	Arg65Trp	rs139468527	Pathogenic	0.0019	EDS, classic type, 1	Disease-causing
	COL5A1	Collagen type V α1 chain	Val172Phe	rs150147262	VUS	0.0006	EDS, classic type, 1	
14	COL4A3	Collagen type IV α3 chain	Leu1474Pro	rs200302125	Pathogenic	0.0046	Alport syndrome, ar	Disease-causing
	COL4A4	Collagen type IV α4 chain	Gly972Arg	rs767704202	Pathogenic	<0.0001		
16	COL5A2	Collagen type V α2 chain	Pro1103Leu	rs150401168	VUS	<0.0001	EDS, classic type, 2	Suggestive
	COL5A1	Collagen type V α1 chain	Thr1757Met	rs2229817	VUS	0.0019	EDS, classic type, 1	
r-CeAD								
19	COL3A1	Collagen type III α1 chain	Lys1313Arg	rs111840783	VUS	0.0013	EDS, type 4	Suggestive
23	ABCC6	ATP binding cassette subfamily C member 6	Arg1141Stop	rs72653706	Pathogenic	0.0021	Arterial calcification of infancy, 2	Suggestive
26	COL5A2	Collagen type V α2 chain	Gly213Ser	rs753789459	Pathogenic	<0.0001	EDS, classic type, 2	Suggestive
27	RNF213	Ring finger protein 213	Trp1231Leu	rs61741961	Pathogenic	0.0151	Moyamoya disease 2, susceptibility to	Suggestive
28	RNF213	Ring finger protein 213	His1231Asp	not in dbSNP	Pathogenic	<0.0001	Moyamoya disease 2, susceptibility to	Suggestive
32	ABCC6	ATP binding cassette subfamily C member 6	Arg1141Stop	rs72653706	Pathogenic	0.0021	Arterial calcification of infancy, 2	Suggestive

Note: Variants were considered pathogenic if assigned 'pathogenic' in the ClinVar database or if pathogenicity was predicted by Polyphen-2 as well as by SIFT. The allele frequency of the variants was assessed for the European (non-Finnish) population of the ExAC database. Associated phenotypes were found in the OMIM database. Pathogenic findings in genes associated with arterial aneurysm or dissection were considered as 'disease-causing'. Variants of unknown significance (VUS) in genes associated with arterial aneurysm or dissection and pathogenic variants in genes associated with other vascular conditions were considered as 'suggestive'.

AF: allele frequency; EDS: Ehlers–Danlos syndrome; HANAC: hereditary angiopathy with nephropathy, aneurysms, and muscle cramps.

background and suggest that the identification of CeAD-causing genetic variants is more likely in f-CeAD than in r-CeAD patients.

In our study, suggestive variants were only identified if they (1) were pathogenic; (2) occurred in at least two different families and (3) were associated with a vascular phenotype. As a consequence, risk variants, sporadic variants occurring in a single family only, or variants without known phenotypes could not be identified in the current study. It is therefore likely that analysis of a larger study sample using the same criteria would lead to the identification of further gene variants suggestive of or causing CeAD.

In the current study sample, two patients each had two amino acid substitutions in type V collagens (COL5A1, COL5A2), which confirms earlier observations.[16,17] Two mutations affecting one and the same collagen type were also found in monozygotic twins with digenic Alport syndrome. These findings suggest a joint contribution of several genetic variants in the pathogenesis of CeAD. The present study, however, aimed at identifying a single, rare, deleterious gene variant in each pedigree and was not designed to explore gene–gene interactions or pathophysiological pathways. The heterogeneity of the findings in the current sample is striking and in agreement with previous genetic studies.[17,18] Similarly, electron microscopic studies of dermal connective tissue alterations in CeAD patients pointed to the existence of different types of morphologic alterations with similarity to findings in vascular Ehlers–Danlos syndrome, classic Ehlers–Danlos syndrome, or heterozygote carriers of Pseudoxanthoma elasticum (PXE).[19] The identified variants in COL3A1 (vascular EDS), COL5A1/2 (classic EDS) and ABCC6 (PXE) are known genes associated with these connective tissue disorders. As electron microscopic investigation of skin biopsies was not performed in our patients, we were not able to study whether the presence of such genetic variants was associated with corresponding ultrastructural abnormalities of the skin.

Heterogeneity of genetic findings was also observed in a recent study of Chinese patients with intracranial vertebral-basilar artery dissection that identified pathogenic variants in COL3A1, FNB1 and TNXB.[20] Our findings show also overlap with studies of patients with coronary artery dissection with pathogenic variants in COL3A1, FBN1, PKD1 and SMAD3[21,22] and aortic dissection with pathogenic findings in FBN1, ACTA2, and MYH11, COL3A1, TGFBR2 or SMAD3.[23–25] In all these studies, the diagnostic yield is modest with significant molecular findings in about 10% of the patients and with variants of unknown significance in another 10%. In our sample of CeAD patients, we observed a similar genetic heterogeneity, and a slightly higher proportion of genetic findings, possibly due to the selection of the patients.

Compared to our previous pilot study of f-CeAD patients,[8] we expanded our analyses by adding further r-CeAD and f-CeAD patients and by analysing a larger panel of vascular genes followed by a genome-wide exploration of exome variants. Nevertheless, we are aware that the current genetic analysis was not exhaustive. Reasons for missing disease-causing variants may include the application of too stringent prioritisation of variants (with regard to frequency and pathogenicity and occurrence in at least two different pedigrees), the presence of disease-causing variants outside the coding sequences covered by the current exome sequencing technology or the occurrence of copy number variants that are not detected in the current investigation, but that are known to be associated with CeAD including f-CeAD.[7,26] The study sample was selective with the aim of an enriched yield of CeAD-causing variants. The current investigation suggests that f-CeAD patients were enriched for disease-causing variants, whereas CeAD-causing variants were not found in patients with recurrent events. A somewhat similar observation was made in a study of stroke monogenic disorders, suggesting that a family history is an important predictor for finding disease-causing mutations.[27]

We are also aware, that our approach to specifically focus on f-CeAD and r-CeAD patients led to a small sample size of our study which limits the generalisability of our findings and also renders a consistent correlation of genetic findings to clinical phenotypes unfeasible. Further, larger studies are needed to confirm and expand our findings and to allow for a comprehensive analysis of genetic findings in the context of clinical and vascular phenotypes of CeAD patients.

Conclusion

In conclusion, although limited by a small sample size, our findings underscore the role of genetic alterations in CeAD pathogenesis in patients with familial CeAD, whereas their role in recurrent but non-familial CeAD remains to be determined. Our findings add value particularly with regard to individualised counselling of patients with familial CeAD. Further studies are warranted for confirmation and extension of our findings.

Declaration of Conflicting Interests

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Informed consent/ethical approval

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Guarantor

CT and STE.

Contributorship

CT and MK designed/conceptualised the study, analysed/interpreted the data, collected data and drafted the manuscript. STE initiated, designed, conceptualised and supervised the study, analysed/interpreted the data, revised the manuscript, and collected data. All authors: data collection, critical review of the manuscript, editing manuscript for content. All authors agreed on submission of the present version of the manuscript.

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