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Donner, Iikki

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NEXT-GENERATION SEQUENCING IN LARGE PEDIGREE SEGREGATING VISCERAL ARTERY ANEURYSMS SUGGESTS POTENTIAL ROLE OF COL4A1/COL4A2 IN DISEASE ETIOLOGY

Iikki Donner[1]; Ph.D.; iikki.donner@helsinki.fi

Lauri J. Sipilä[1]; M.Sc.; lauri.sipila@helsinki.fi

Roosa-Maria Plaketti[1]; B.Sc.; roosa.plak@gmail.com

Anna Kuosmanen[1]; Ph.D.; anna.kuosmanen@helsinki.fi

Linda Forsström[1]; Ph.D.; linda.m.forsstrom@gmail.com

Riku Katainen[1]; Ph.D; riku.katainen@helsinki.fi

Outi Kuismin[2]; M.D., Ph.D.; outi.kuismin@ppshp.fi

Mervi Aavikko[1, 3]; Ph.D.; mervi.aavikko@helsinki.fi

Pekka Romsi[4]; M.D., Ph.D.; pekka.romsi@ppshp.fi

Juho Kariniemi[5]; M.D., Ph.D.; juho.kariniemi@pphsp.fi

Lauri A. Aaltonen[1]; M.D., Ph.D.; lauri.aaltonen@helsinki.fi

Corresponding author:

Lauri A. Aaltonen; Biomedicum Helsinki, P.O. Box 63 (Haartmaninkatu 8), FI-00014 University of Helsinki, Helsinki, Finland; tel: +358-2941 25595; fax: +358-2941 25610; e-mail: lauri.aaltonen@helsinki.fi

[1] Department of Medical and Clinical Genetics, Medicum, University of Helsinki, Helsinki, Finland

and

Genome-Scale Biology Research Program, Research Programs Unit, University of Helsinki, Helsinki, Finland.

[2] Department of Clinical Genetics, Oulu University Hospital, Oulu, Finland

and

PEDEGO Research Unit, Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland.

[3] Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki, Helsinki, Finland

[4] Department of Vascular Surgery, Oulu University Hospital, Oulu, Finland

[5] Department of Radiology, Oulu University Hospital, Oulu, Finland

ABSTRACT

Background

Visceral artery aneurysms (VAAs) can be fatal if ruptured. Although a relatively rare incident, it holds a contemporary mortality rate of approximately 12%. VAAs have multiple possible causes, one of which is genetic predisposition. Here, we present a striking family with seven individuals affected by VAAs, and one individual affected by a visceral artery pseudoaneurysm.

Methods

We exome sequenced the affected family members and the parents of the proband to find a possible underlying genetic defect. As exome sequencing did not reveal any feasible protein-coding variants, we combined whole-genome sequencing of two individuals with linkage analysis to find a plausible non-coding culprit variant. Variants were ranked by the deep-learning framework DeepSEA.

Results

Two of seven top-ranking variants, NC_000013.11:g.108154659C>T and NC_000013.11:g.110409638C>T, were found in all VAA-affected individuals, but not in the individual affected by the pseudoaneurysm. The second variant is in a candidate cis-regulatory element in the fourth intron of *COL4A2*, proximal to *COL4A1*.

Conclusions

As type IV collagens are essential for the stability and integrity of the vascular basement membrane and involved in vascular disease, we conclude that *COL4A1* and *COL4A2* are strong candidates for VAA susceptibility genes.

INTRODUCTION

Aneurysms are caused by a local weakening of the arterial wall (medial degeneration). True aneurysms involve all three layers (intima, media, and adventitia) of the wall, and are defined as a 50% increase in the normal diameter of the vessel. They can be saccular or fusiform. Saccular aneurysms involve only a portion of the vessel wall and are spherical in shape with a narrow stem. A fusiform aneurysm is spindle shaped as it bulges out on all sides, forming a dilated artery. In contrast to true aneurysms, pseudoaneurysms lack arterial wall components: they are contained ruptures lined by surrounding tissue.

Visceral artery aneurysms (VAAs) include aneurysms of the splenic, hepatic, superior mesenteric, gastric, celiac, pancreaticoduodenal, gastroduodenal, inferior mesenteric, and renal arteries.[1] Renal artery aneurysms are sometimes considered separately due to a modest difference in etiology.[2] The incidence of VAAs in the general population has been reported to be 0.01-2%.[3] Among the most common sites are the splenic, renal, and hepatic arteries.

VAAs may have multiple causes, and these vary slightly depending on the affected artery. The main etiology is often cited as atherosclerosis—a disease caused by the formation of plaque inside the arteries, causing them to narrow and harden—although there is reason to believe it only has a secondary role.[3] Hypertension and connective tissue disorders are also

often associated with VAAs.[4] Of the connective tissue disorders, Marfan syndrome, Ehlers-Danlos syndrome (EDS), and fibromuscular dysplasia (FMD) are most commonly cited to predispose to aneurysm formation. Marfan syndrome is an autosomal dominant genetic disorder of connective tissue caused mainly by mutations in *FBN1*.[5–7] The protein product of the gene, fibrillin-1, provides force-bearing structural support to connective tissue throughout the body.[8] EDS is a heterogeneous entity consisting of heritable connective tissue disorders. The vascular type of EDS results from mutations in *COL3A1.*[9] Of all EDS types described, it is most prone to vascular complications including aneurysm formation and rupture. FMD is defined as an idiopathic, non-inflammatory and non-atherosclerotic disease of arterial wall musculature that leads to stenosis of small and medium-sized arteries. It most commonly affects the renal and carotid arteries.[10] Up to 90% of FMD patients are female, and 10% have an affected first-degree relative.[11]

The etiology of pseudoaneurysms differs from that of true aneurysms, as they are most often caused by trauma (also iatrogenic) and inflammation, in particular chronic pancreatitis.

Aneurysms are often asymptomatic but carry a risk of rupture. A ruptured VAA can be fatal due to uncontrolled bleeding. At present the mortality rate is approximately 12%, however, this varies depending on the location of the lesion.[12] Splenic artery aneurysms are up to four times more common in women and linked to multiparity.[13] A probable cause is the increase in blood flow in combination with hormone-induced changes in arterial composition during pregnancy. Importantly, pregnant women have an increased risk of aneurysm rupture, and the mortality rate of a ruptured splenic artery aneurysm during pregnancy is approximately 75% for the mother and 95% for the fetus, compared to 25% in the general population.[14]

Collagen type IV alpha 1 and collagen type IV alpha 2 molecules (encoded by *COL4A1* and *COL4A2*, respectively) make up type IV collagen. Heteromers consisting of two alpha-1 chains and one alpha-2 chain attach to each other to form complex protein networks that are the main component of basement membranes. Mutations in these genes cause rare multi-system disorders, characterized by abnormal blood vessels, ocular dysgenesis, myopathy and renal pathology.[15–22] The effects of mutations in the mouse orthologs *Col4a1* and *Col4a2* have been studied extensively.[16, 23–25] Complete deficiency of both proteins is embryonic lethal and associated with neuronal ectopias, disorganization of the capillary network during angiogenesis and impaired placental development, whereas double heterozygosity of the null alleles lacks an obvious phenotype.[23] However, mice heterozygous for certain missense and splice-site mutations exhibit diverse effects, such as ocular, renal, pulmonary, muscular, vascular, reproductive, and central nervous system disorders, consistent with the pleiotropic effects linked to heterozygous human *COL4A1*/*COL4A2* mutations.

Here, we present a large family with seven individuals affected by visceral artery aneurysms. Multiparity is associated with this type of aneurysms, splenic in particular, and all affected individuals were multiparous. As the number of affected individuals is nevertheless striking, we DNA sequenced the family members in an attempt to find a possible underlying genetic defect.

MATERIALS AND METHODS

Ethics statement

This research was approved by the National Institute for Health and Welfare and the ethics committees of the hospital districts of Helsinki and Uusimaa and North Ostrobothnia. All sequenced individuals gave informed written consent. Use of archival tissue was approved by the National Supervisory Authority for Welfare and Health (Valvira).

Patients

The proband (II:1) had several renal artery aneurysms and her splenic artery was dilated. She had altogether six siblings, three of whom were female and affected by aneurysms (**Figure 1**). II:2 and II:4 had multiple splenic and renal artery aneurysms, whereas II:3 had a splenic artery aneurysm and a small internal carotid artery aneurysm. All sisters were multiparous with six to 15 children each. Three women in the next generation also had aneurysms; III:1 had two splenic artery aneurysms, III:2 had an internal carotid artery aneurysm, and III:3 had several splenic artery aneurysms and a celiac artery lesion. Again, all were multiparous, and the number of children was two to nine. Patient vascular findings are listed in **Table 1**.

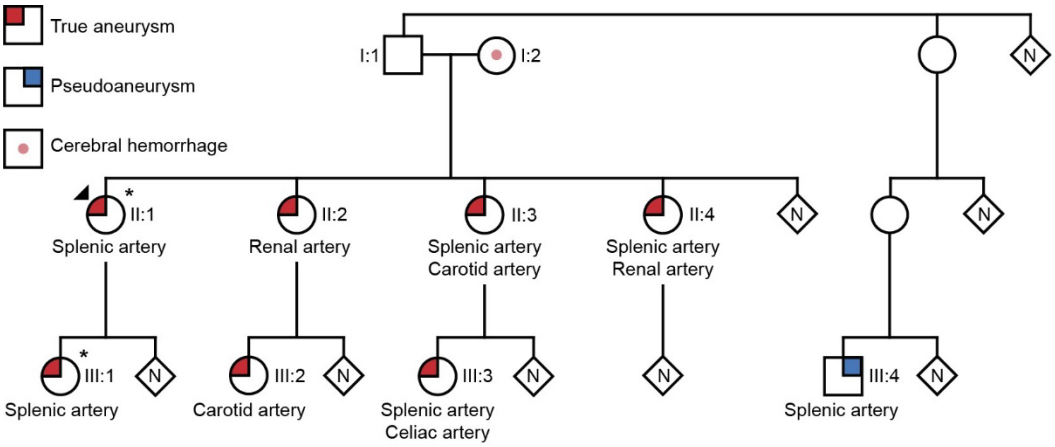

Figure 1. Affected individuals in the pedigree and type/s of aneurysm diagnosed. Individuals marked with an asterisk were genome sequenced. Circles represent females, squares represents males, and diamonds represent individuals of undisclosed sex. The full pedigree is not depicted and the sex of most unaffected individuals is not disclosed due to reasons of confidentiality.

Table 1. Patient vascular imaging and findings.

Patient	Procedure	Area	Findings
I:2	DSA	carotid artery and vertebral artery	possible middle cerebral artery aneurysm
II:1	CTA	splenic artery	five splenic artery aneurysms
	MRI and 3D TOF MRA	head	no aneurysms
	CTA	aorta	resected spleen, left renal artery aneurysm, splenic artery aneurysm
	CTA	coronary arteries	no aneurysms
	MRI	upper abdomen	no aneurysms
II:2	CT	abdomen	two left renal artery aneurysms, right renal artery aneurysm, localized fusiform dilatation of splenic artery
	MRA	cranial vasculature	hypoplastic anterior communicating artery
II:3	CTA	cerebral arteries	internal carotid artery aneurysm/localized fusiform dilatation
	CT	upper abdomen	splenic artery aneurysm
	CTA	abdominal aorta and groin	same finding as above
II:4	CTA	aorta	three splenic artery aneurysms, localized fusiform dilatation of splenic artery, accessory renal arteries (patient has altogether five renal arteries)
	CTA	abdominal aorta	same findings as above and right renal artery aneurysm
	MRI	head	no aneurysms
III:1	CTA	abdominal aorta, groin, and visceral arteries	two splenic artery aneurysms
	3D TOF MRA	cranial vasculature	no aneurysms
III:2	MRA	cranial vasculature	left internal carotid artery aneurysm/localized fusiform dilatation
	MRA	thoracic and abdominal aorta	no aneurysms
III:3	CTA	abdominal aorta and groin	two splenic artery aneurysms, localized fusiform dilatation of the celiac artery, accessory renal artery
	CTA	cranial vasculature	no aneurysms
	3D TOF MRA	cerebral arteries	no aneurysms
	MRA	thoracic and abdominal aorta	no aneurysms
III:4	Endovascular embolisation for bleeding with radiological guidance	splenic artery	splenic artery pseudoaneurysm, abnormal elongation of femoral artery

In addition to their aneurysms, II:2 had structural damage to the posterior communicating artery, II:2 and III:3 had accessory renal arteries, and II:1, II:2, and III:3 had hepatic hemangiomas.

The mother of the proband (I:2) had had a cerebral hemorrhage of unknown cause. A male paternal second cousin of the proband had presented with a ruptured splenic artery pseudoaneurysm and exhibited abnormal elongation of the femoral artery. None of the patients exhibited clinical features of Marfan syndrome.

Methods

DNA was extracted from blood with conventional methods. Exome sequencing was performed on affected patients and the parents of the proband with Illumina HiSeq 4000 (Illumina, San Diego, CA). Whole genome sequencing was performed on one patient from generation II and one from generation III (**Figure 1**) with Illumina HiSeq X Ten (Illumina, San Diego, CA). We performed non-parametric linkage analysis of the sequenced family members using MERLIN and the Kong & Cox exponential model.[26,27] Variant annotation and filtering were performed with BasePlayer, a variant analysis and data integration platform.[28]

Coding variants were required to be found in all individuals affected with true aneurysms (seven cases) and have a MAF < 0.01 in gnomAD exomes and gnomAD exomes' Finnish subpopulation. Non-coding variants were required to be found in both genome-sequenced individuals, and have a MAF < 0.005 in gnomAD genomes, and a MAF < 0.01 in 373 in-house control genomes. We also required the non-coding variants to localize to areas with a LOD > 0.5. LOD scores had been computed previously.

To rank variants, we used the standalone version of DeepSEA, a deep learning-based algorithmic framework for predicting chromatin effects of sequence alterations.[29] DeepSEA ranks SNVs only, large structural variation such as copy number data is not used in training the framework. The filtered variants from the previous step, 799 in total, were used as input. Variants were prioritized based on their HGMD probability and functional significance scores. We chose to validate the five best scoring variants in both groups. In total, 7 high-scoring variants, listed in **Table 2**, were validated by Sanger sequencing.

Table 2. Top-scoring variants chosen for Sanger validation.

hg38	HGVS	funsig*	HGMD*
1:58713768	NC_000001.11:g.58713798_58713907del	0.008	6.47E-07
4:6762013	NC_000004.12:g.6762027_6762028insCGAGGAGGCGGGCAG	0.01	0.69
4:25919954	NC_000004.12:g.25919954C>T	0.044	0.78
7:156992634	NC_000007.14:g.156992634G>A	0.015	0.51
10:117684640	NC_000010.11:g.117684643del	0.025	0.77
13:108154659	NC_000013.11:g.108154659C>T	0.066	0.79
13:110409638	NC_000013.11:g.110409638C>T	0.016	0.7

*as estimated by DeepSEA (Zhou and Troyanskaya 2015); funsig = unsupervised functional significance score, HGMD = probability of being a Human Gene Mutation Database mutation, classifier trained with HGMD data

For a more detailed description of the methods, please refer to the **Supplemental Methods**.

RESULTS AND DISCUSSION

In order to find the possible genetic cause of VAAs in the family, patients were, apart from the individual affected by a pseudoaneurysm, initially exome sequenced. We first searched for rare (MAF < 0.01) variants shared by the affected family members and either parent, with negative results. We next whole-genome sequenced two patients, and searched for candidate

variants segregating between them. Since we had the exomes of seven patients available, we used these data to determine genomic regions likely shared by the affected individuals by linkage analysis. Altogether 799 non-coding variants shared by the two genome-sequenced individuals resided in the regions and passed filtering. The variants were subsequently ranked by the deep-learning model DeepSEA. The presence of the top-ranking variants (**Table 1**) in the exome-sequenced individuals was determined by Sanger sequencing.

None of the variants were found in all patients with true aneurysms and the patient with the pseudoaneurysm. However, two variants—the substitutions NC_000013.11:g.108154659C>T and NC_000013.11:g.110409638C>T—were shared by all patients with true aneurysms. The first variant is located in the second intron of the diverticulitis associated gene *FAM155A*, and the second variant in the fourth intron of *COL42*, a gene linked to cerebrovascular disease (intracerebral hemorrhage, porencephaly, and brain small-vessel disease).[17, 30–32]

FAM155A encodes a relatively poorly described membrane protein. *COL4A2*, on the other hand, encodes collagen type IV alpha 2 extracellular matrix protein, a subunit of type IV collagen, the major structural component of basement membranes. *COL4A1* and *COL4A2* share a common promoter, form heterotrimers (2:1), and make up the majority of type IV collagen.[16] Both contain activating elements which are indispensable for efficient transcription, and the third intron of *COL4A2* also contains a silencing element.[23,33] In addition to being linked to cerebrovascular disease (porencephaly and brain small-vessel disease with hemorrhage), *COL4A1* has also been shown to cause nonsyndromic congenital

cataract, tortuosity of retinal arteries, and hereditary angiopathy with nephropathy, aneurysms, and muscle cramps (HANAC) syndrome.[34–38]

According to ENCODE data, both variants are located in candidate cis-regulatory elements (cCREs; accessions EH38E1696504 and EH38E1697888) with a distal enhancer-like signature.[39] It is thus plausible that they could alter the expression of nearby genes. Due to its proximity to *COL4A1* and *COL4A2*, the variant NC_000013.11:g.110409638C>T is particularly interesting. Besides having already been linked to vascular disease, type IV collagens form complex, covalently linked structural scaffolds that are fundamental for the integrity and function of the basement membrane.[40] The basement membrane provides the vasculature with mechanical support, serves as a diffusion barrier, and also plays a crucial role in signaling events that regulate endothelial cell migration, proliferation, and survival.[41] Altered expression of either *COL4A1* or *COL4A2* could cause this structure to weaken or malfunction. This in combination with additional pregnancy induced changes in arterial wall composition could plausibly have caused the striking incidence of aneurysms seen here, especially as 5/7 affected women were grand multiparous. In conclusion, mutations in the regulatory regions of *COL4A1* and *COL4A2* may alter VAA susceptibility. However, additional studies involving functional and model data are warranted to validate the findings.

Deciphering the genetics behind familial VAAs enables genetic testing, informed decision making, and medical surveillance of at-risk individuals. Considering the generally high mortality rate of aneurysm rupture, not to mention the catastrophic event of rupture during pregnancy, this is of utmost importance.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

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