Published Ahead of Print on October 26, 2020 as 10.1212/WNL.0000000000011125

OPEN

Neurology®

The most widely read and highly cited peer-reviewed neurology journal
The Official Journal of the American Academy of Neurology

AMERICAN ACADEMY OF NEUROLOGY®

Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000011125

ANGPTL6 genetic variants are an underlying cause of familial intracranial aneurysms

Isabel C Hostettler MD[1,2], Benjamin O'Callaghan MSci[2,3], Enrico Bugiardini MD[3,4], Emer O'Connor MD[2], Jana Vandrovcova PhD[4], Indran Davagnanam[5], Varinder Alg MD[1], Stephen Bonner PhD[6], Daniel Walsh PhD[7], Diederik Bulters FRCS[8], Neil Kitchen PhD[9], Martin M Brown FRCP[1], Joan Grieve MD[9], David J Werring PhD FRCP[1], Henry Houlden PhD FRCP[2], on behalf of the Genetics and Observational Subarachnoid Haemorrhage (GOSH) Study investigators

The Article Processing Charge was funded by Wellcome Trust and MRC.

[1] *Stroke Research Centre, University College London, Institute of Neurology, London, UK*

[2] *Neurogenetics Laboratory, The National Hospital of Neurology and Neurosurgery, London, UK*

[3]*MRC Centre for Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London*

[4]*Department of Neuromuscular Disorders, UCL Institute of Neurology, Queen Square, London*

[5]*Department of Neuroradiology, The National Hospital for Neurology and Neuro- surgery, University College London Hospitals NHS Foundation Trust, Queen Square, Lon- don*

[6] *Department of Anaesthesia, The James Cook University Hospital, Middlesbrough, UK*

[7] *Department of Neurosurgery, King's College Hospital NHS Foundation Trust, London, UK*

[8]*Department of Neurosurgery, University Hospital Southampton NHS Foundation Trust, Southampton UK*

[9] *Department of Neurosurgery, The National Hospital of Neurology and Neurosurgery, London, UK*

Title character count: 84

Word count abstract: 241

Word count paper: 2508

Number of references: 28

Number of tables: 2

Number of figures: 5

Corresponding author: Professor Henry Houlden; Email: h.houlden@ucl.ac.uk

Analysis were conducted by Isabel C Hostettler, MD, Benjamin O'Callaghan, MSci, and Jana Vandrovcova, PhD, University College London.

Keywords: 1) Intracranial hemorrhage, 2) Aneurysmal subarachnoid hemorrhage, 3) ANGPTL6, 4) Rare genetic variants, 5) Family history

Study funding:

The GOSH study is funded by the Stroke Association. This work was partly undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. These funding bodies had no direct involvement in this study.

Disclosure:

Isabel C Hostettler and Henry Houlden received funding from the Alzheimer Research UK and Dunhill Medical Trust Foundation.

Duncan Wilson and David J Werring received funding from the Stroke Foundation/British Heart Foundation.

Benjamin O'Callaghan reports no disclosures.

Enrico Bugiardini reports no disclosures.

Emer O'Connor reports no disclosures.

Jana Vandrovcova reports no disclosures.

Indran Davagnanam reports no disclosures.

Varinder Alg reports no disclosures.

Stephen Bonner reports no disclosures.

Daniel Walsh reports no disclosures.

Diederik Bulters reports no disclosures.

Neil Kitchen reports no disclosures.

Martin M Brown reports no disclosures.

Joan Grieve reports no disclosures.

Acknowledgement

We thank the many NIHR Clinical Research Network Practitioners and the investigators at participating centres for their assistance with this study, and especially the patients for taking part.

ABSTRACT

Background and Purpose: To understand the role of the angiopoietin-like 6 gene (ANGPTL6) in intracranial aneurysms (IA) we investigated its role in a large cohort of familial IAs.

Methods: Inclusion of individuals with family history of IA recruited to the Genetic and Observational Subarachnoid Haemorrhage (GOSH) study. The ANGPTL6 gene was sequenced using Sanger sequencing. Identified genetic variants were compared to a control population.

Results: We found six rare ANGPTL6 genetic variants in 9/275 individuals with a family history of IA (3.3%), none of them were present in controls: Five missense and one nonsense mutation leading to a premature stop codon. One of these had been previously reported: c.392A>T (p.Glu131Val) on exon 2, another was very close: c.332G>A (p.Arg111His). Two further genetic variants lie within the fibrinogen-like domain of the ANGPTL6 gene, which may influence function or level of the ANGPTL6 protein. The last two missense mutations lie within the coiled-coil domain of the ANGPTL6 protein. All genetic variants were well conserved across species.

Conclusion: ANGPTL6 genetic variants are an important cause of IA. Defective or lack of ANGPTL6 protein is therefore an important factor in blood vessel proliferation leading to IA; dysfunction of this protein is likely to cause abnormal proliferation or weakness of vessel walls. With these data, not only do we emphasise the importance of screening familial IA cases for ANGPTL6 and other genes involved in IA, but also highlight the ANGPTL6 pathway as a potential therapeutic target.

Classification of Evidence:

This is a Class III study showing some specificity of presence of the ANGPTL6 gene variant as a marker of familial intracranial aneurysms in a small subset of those with familial aneurysms.

INTRODUCTION

Three percent of the general population harbour an unruptured intracranial aneurysm (UIA)[1]. Ruptured UIA cause aneurysmal subarachnoid haemorrhage (aSAH), a devastating condition with high mortality and morbidity and great socio-economic burden[2, 3] ENREF 4. Despite efforts in discovering factors associated with aneurysm formation and rupture, little is known about mechanisms driving aneurysm formation, growth and rupture as well as the genetic background of this disease. Some inherited disorders, such as autosomal dominant polycystic kidney disease, have been associated with increased aneurysm formation as well as rupture[4, 5] ENREF 6. Increasing evidence exists that genetic factors also play a role in IA formation in patients without underlying genetical diseases[6, 7].

Most IA are acquired lesions arising under haemodynamic stress and associated defective wall response[8]. Disturbances in angiogenic factors can lead to changes in vessel structure and cerebral artery wall stability. Genes involved in angiogenesis have therefore recurrently been investigated. The angiopoietin-like 6 (ANGPTL6) gene is a protein coding gene belonging to the ANGPTL family, which have been associated with regulatory capacities in angiogenesis[9-11]. ANGPTL6 has been identified as a circulating pro-angiogenic factor increasing endothelial permeability[9, 12]. A recent publication found significant enrichment in rare coding variants within the ANGPTL6 in patients with familial IA[13]. They demonstrated a reduction of 50% in the serum concentration of ANGPTL6 in heterozygous individuals for c.1378A>T[13].

We aimed to externally validate the previous findings, to search for additional rare variants and to evaluate the rate of rare variants in a large cohort of individuals with family history of IA.

METHODS

Selection of patients

For the main analysis we included patients with available DNA and familial IA, where at least one affected first or second degree relative had IA who were recruited into the Genetic and

Observational Subarachnoid Haemorrhage (GOSH) study to also evaluate whether previously reported variants would be detected in those with only one affected first- or second-degree family member as well. (Figure 1). Only one patient had only one affected first or second degree relative. Both, patients with unruptured IA and ruptured IA could be included in this study. We compare patients with familial IA and the rest of the cohort in Table 1 in order to highlight differences. A description of the whole cohort is published elsewhere[14]. Written informed consent was obtained from all participants, or in case of lack of capacity from a representative. We did not include patients with peri-mesencephalic SAH (defined by the blood distribution mainly or only in the cisterns around the midbrain), absence of intracranial aneurysm and SAH due to trauma as well as mycotic aneurysms. None of the patients included had been diagnosed with either Ehlers Danlos, Marfan syndrome or polycystic kidney disease. IA was confirmed on CT/MR angiography or digital subtraction angiography. As the primary aim of this study was to replicate the association between nIA and identifier variants in ANGPTL6, and genetic data from affected/unaffected relatives was not collected with the exception of a mother and daugther pair, segregation analysis was not performed.

The study was approved by the corresponding Ethics committee (reference number: 09/H0716/54).

We used brain biopsy controls from our own laboratory as well as the MRC brain exome controls, excluding individuals with known previous stroke (n=59), to compare the frequency of rare genetic variants found in cases with a control cohort.

The primary research question is to externally validate and evaluate additional rare variants in the ANGTL6 gene. According to the Classification of Evidence this is a Class III evidence study.

Sanger Sequencing

We extracted genomic DNA and performed Polymerase-Chain Reaction (PCR) to amplify exons 2-6 of the ANGPTL6 gene followed by Sanger sequencing. Primer sequences can be provided upon request. We cleaned up the PCR product using Exo-Fast, before performing dye-terminator

sequencing PCR with BigDye Terminator v3.1 (Thermofisher). The sequencing PCR product was cleaned using Sephadex (Sigma Aldrich) before being loaded onto the AB1 3730xl genetic analyser (Applied Biosystems®, Foster City, CA, USA). We used SeqScape v.3.0 software (Applied Biosystems) for sequence analysis.

In accordance with the previous publication we filtered out variants with a minor allele frequency (MAF) higher than 1% in the Genome Aggregation Database (gnomAD) database, v2.1.1[13].

Brain bank neuropathologically normal and MRC Controls

Libraries for brain biopsy controls were generated using TruSeq Exome Enrichment Kit (Illumina), according to the manufacturer's protocol and sequenced on Illumina HiSeq generating 150bp paired end reads. The average coverage obtained was 36x. Reads were aligned to GRCh37 using Novoalign and variants were called using GATK HaplotypeCaller. Whole exome sequencing data from the MRC brain bank samples were obtained from EGA (EGAS00001001599), which were mainly sequenced using the Illumina HiSeq 2000 array (https://ega-archive.org/).

We compared the MAF of rare variants in our cases with our in-house brain control exomes, the MRC controls and data available on population databases, specifically gnomAD (https://gnomad.broadinstitute.org) and ExAC (http://exac.broadinstitute.org) (Figure 2)[15]. We then further checked the estimated effect of the according rare mutation using different computation predictive programs. As functional prediction scores we used PolyPhen-2 and SIFT. These are used to help interpret the sequence variant.

Standard Protocol Approvals, Registrations, and Patient Consents

This study received approval from the ethical standards committee on human experimentation for any experiments using human subjects (UKCRN ID-7512, 09/H0716/54).

Data Availability

Anonymized data will be shared by request from any qualified investigator. EGAS00001001599 can be requested from the European Genome-phenome Archive.

RESULTS

We included 275 patients with family history of IA. Of these 275 patients, 185 (67.3%) had suffered from an aSAH. Baseline characteristics and risk factors are summarized in Table 1. Comparing familial with sporadic IA we found that individuals with familial IA were as expected younger than their non-familial equivalent. Interestingly, individuals with familial IA were more frequently female, were more frequently on oral anticoagulation, but not on antiplatelets. We did not find a difference in hypertension. In our cohort of patients with family history of IA, 9/275 individuals (3.3%) harboured a rare (<0.01% gnomAD MAF) exonic mutation (see Figure 2 for the family trees of 7/9 individuals).

We found a total of 13 different nucleotide changes in ANGPTL6. Four of these changes resulted in the synonymous amino acid and three had a gnomAD MAF above 1% (two of which were also present in the control group) and were consequently not considered. In the remaining 6 rare genetic variants, we had 5 different missense mutations and one nonsense mutation (Table 2). None of these rare genetic variants were present in our control population of 1800 Individuals. Figure 3 demonstrates imaging studies of the aneurysms of three of your patients.

Previously reported variants and associated variants

We replicated the previous finding of c.392A>T (p.Glu131Val), which has previously been reported as deleterious as per SIFT[13]. In our cohort this mutation was present in 2 individuals (MAF 0.004; Figure 2 individuals 1 and 2). It was not present in our control population or the ExAC database, however it was present in gnomAD population controls with a MAF of 0.001. p.Glu131Val is moderately conserved across species, and lies within the linker region between the coiled-coil and fibrinogen-like domains (FLD), but no functional role has been described so far (Figure 4 and 5). We found a previously not reported mutation which is very close to c.392A>T: c.332G>A (p.Arg111His) in 1 individual (MAF 0.002; Figure 2 individual 8). This variant was not present in either our control population, ExAC or gnomAD databases and is estimated to be

deleterious as per SIFT. The mutation lies within the coiled-coil domain which is important for the function of angiopoietin-like proteins[16]. p.Arg111His is moderately conserved across species but no functional role for this specific residue has been described.

Novel rare variants

In addition to c.332G>A (p.Arg111His), which lies in close proximity to the previously reported c.392A>T (p.Glu131Val), we found 4 additional rare genetic variants which have not been reported. Most notably, we found a c.851C>G (p.Ser284*) nonsense mutation leading to a premature stop codon in one individual (MAF 0.002; Figure 2 individual 3). This variant was not present in our own control cohort; however, it was present in ExAC and gnomAD with a MAF of 0.0004 and 0.0005 respectively. This mutation occurs in Ex4 and is predicted to lead to nonsense-mediated decay (NMD) and/or production of a truncated protein lacking the majority of the FLD. This stop-gain mutation has been previously described to result in complete loss of secretion of ANGPTL6 protein in cells[17]. It is moderately conserved across species.

Another mutation present in our cases lying within the FLD of the ANGPTL6 gene is c.1073G>A (p.Arg358His), which was again present in one individual (MAF 0.002; Figure 2 individual 4). This variant was not present in our control group but present in ExAC and gnomAD databases with low MAF of 0.00002 and 0.00003 respectively. p.Arg358His lies within the FLD which is also important for the activity of angiopoietin-like proteins and is moderately conserved across species, but no functional role of this specific residue has been described[16].

Finally, we found two further missense mutations: c.243G>C (p.Arg81Ser) in one individual (MAF 0.002; Figure 2 individual 5) and c.245T>A (p.Gln82Leu) in three individuals (MAF 0.005; Figure 2 individuals 6 and 7, family tree for the third individual not available). Interestingly, 2 of these individuals were related (mother and daughter). These two genetic variants affect adjacent residues, and both lie within the coiled-coil domain of ANGPTL6. Both amino acids are moderately conserved across species but for neither of them has a functional role been previously described.

DISCUSSION

We present a study of the ANGPTL6 gene in a large cohort with family history of IA replicating one of the previously presented variants: c.392.A>T (p.Glu131Val) in the linker region. Additionally, we found c.332G>A (p.Arg111His) located very close to this previously described mutation within the coiled-coiled domain. We also found two genetic variants in the FLD of the ANGPTL6 gene, one being a nonsense mutation that leads to a stop-gain which has been previously described to result in complete loss of ANGPTL6 secretion. None of the here presented genetic variants were found in our 1800 controls.

Most likely due to the continuous expansion and sometimes merging of databases such as gnomAD and ExAC, some of the previously described variants have now also been described in these control populations. We did not replicate any of the other described variants but found 4 additional ones. All of the here reported rare variants were, if present at all, much rarer in the analysed control populations.

Our study provides additional insight, building up on the previous study on ANGPTL6 in individuals with familial IA[13]. The c.851C>G (p.Ser284*) stop-gain mutation we identified, lies in ANGPTL6 Ex4 and likely results in NMD of the mutant transcript which could contribute to haploinsufficiency. It is also possible that this mutation leads to the expression of a truncated protein lacking the majority of the FLD, reducing ANGPTL6 protein function and/or stability. In fact, a previous study has reported this variant leads to a complete loss of secretion of the ANGPTL6 protein in cells[17]. The actual effect of reduced ANGPTL6 secretion remains unknown and warrants further investigation. We also identify novel genetic variants in the FLD and coiled-coil domain of ANGPTL6 which might be impairing the pro-angiogenic function and/or overall stability of the protein[16]. Although no function specific to ANGPTL6 has been established yet, the FLD is suggested to regulate angiogenic activities[10, 16]. The same is true for the coiled-coil domain. Several functions of this domain have been reported, including activity in polymer

formation, molecular recognition, cytoskeletal regulation, and pH sensing[18]. Based on the information available, a dominant negative mechanism cannot be conclusively ruled-out, especially in missense changes. A potential mechanism could be that the mutation alters the protein structural binding, preventing ANGPTL6 signaling. The ANGPTL family also regulates lipid and glucose metabolism and by doing so indirectly influencing risk factors for IA formation such as hypertension[19-22].

As Bourcier et al. hypothesized, individuals with rare variants in the ANGPTL6 gene might require additional factors in order to trigger the development of IA[13]. Therefore, heterozygous ANGPTL6 genetic variants might only exhibit a deleterious effect in combination with certain risk factors such as high blood pressure. Other genes have also been previously reported to play a role in familial IA such as ADAMST15 and PCNT. ADAMST 15 has been shown to be significantly aggregated in families with IA[23]. It might be associated with IA formation through abnormal transcription of metalloproteinases[24]. Two rare variants of the PCNT gene have previously been found in familial IA cases[25]. Deletions and mutations of this gene a type of dwarfism which is association with IA in up to 20%[25]. This indicates the genetical complexity of this disease.

Our study has strength: we present a large cohort of individuals with family history of IA sequenced using Sanger sequencing. Sanger sequencing remains the gold standard for mutation confirmation. What's more, concordance between Sanger sequencing and whole-exome sequencing has been reported to be 97.3%[26]. This allowed us to check the variants found in our cases in a control population sequenced by whole-exome sequencing. None of the rare genetic variants found in our cases were present in our own control dataset; if the genetic variants were present in the gnomAD and ExAC control populations they were significantly rarer, reducing the likelihood for winner's curse.

Our study has limitations: we did not sequence either affected nor unaffected family members (expect for two individuals who were related) as DNA for family members was not available and our aim was to externally validate the previous results of 4 rare coding variants within the

ANGPTL6 gene; information and DNA for relatives were generally not collected as part of the GOSH study. This would be especially of interest as one variant was present in both mother and daughter indicating a potential familial clustering. We included patients with only one affected second degree relative not fulfilling the classical criteria of familial IA. We do not believe that this limits our results as none of the patients harbouring a rare variant only had one affected second-degree relative. Additionally, we were not able to obtain the family trees in two individuals; one of whom was deceased (c.245T>A; p.Gln82Leu) and the other one was not contactable (c.332G>A; p.Arg111His). We can therefore not make any statements about penetrance in our population. Although we cannot make any statements about penetrance and we refrained from burden analysis due to cases and controls not having been sequenced with the same method, we demonstrated that the rare genetic variants found in our cases are far less frequent or even absent in the considered control cohorts. Therefore, a genetic contribution to this disease, including by rare variant in the ANGPTL6 gene, is highly likely.

CONCLUSION

In summary, we replicated some of the previously reported genetic variants in the ANGPTL6 gene in a large cohort of individuals with family history of IA. Novel found genetic variants support ANGPTL6 loss of function might be a genetic risk factor influencing IA development. These findings however remain to be confirmed in an independent cohort. The function of the ANGPTL6 gene remains to be further evaluated. It might offer a target for screening familial IA cases for ANGPTL6 as well as other genes involved in IA, but also highlight the ANGPTL6 pathway as a potential therapeutic target.

APPENDIX 1: AUTHORS

Name	Location	Contribution
Isabel C Hostettler	Stroke Research Centre, University College London, Institute of Neurology, London, UK	Design and conceptualized study; Acquisition of data; analysed the data; drafted the manuscript; revised the manuscript
Benjamin O'Callaghan	Neurogenetics Laboratory, The National Hospital of Neurology and Neurosurgery, London, UK	Contributed to the laboratory work; analysed the data; drafted the manuscript; revised the manuscript
Enrico Bugiardini	Neurogenetics Laboratory, The National Hospital of Neurology and Neurosurgery, London, UK	Contributed to the laboratory work; analysed the data; drafted the manuscript; revised the manuscript
Emer O'Connor	Neurogenetics Laboratory, The National Hospital of Neurology and Neurosurgery, London, UK	Contributed to the laboratory work; analysed the data; drafted the manuscript; revised the manuscript
Jana Vandrovcova	Department of Neuromuscular Disorders, UCL Institute of Neurology, Queen Square, London	Analysed the data; interpreted the data;revised the manuscript for intellectual content
Indran Davagnanam	Department of Neuroradiology, The National Hospital for Neurology and Neurosurgery, University College London Hospitals NHS Foundation Trust, Queen Square, London	Analysed the data; revised the manuscript for intellectual content
Varinder Alg	Stroke Research Centre, University College London, Institute of Neurology, London, UK	Acquisition of data; revised the manuscript for intellectual content
Stephen Bonner	Department of Anaesthesia, The James Cook University Hospital, Middlesbrough, UK	Acquisition of data; revised the manuscript for intellectual content
Daniel Walsh	Department of Neurosurgery, King's College Hospital NHS Foundation Trust, London, UK	Acquisition of data; revised the manuscript for intellectual content
Diederik Bulters	Department of Neurosurgery, University Hospital Southampton NHS Foundation Trust, Southampton UK	Acquisition of data; revised the manuscript for intellectual content
Neil Kitchen	Department of Neurosurgery, The National Hospital of Neurology and Neurosurgery, London, UK	Acquisition of data; revised the manuscript for intellectual content
Martin M Brown	Stroke Research Centre, University College London,	Acquisition of data; revised the manuscript for intellectual

	Institute of Neurology, London, UK	content
Joan Grieve	Department of Neurosurgery, The National Hospital of Neurology and Neurosurgery, London, UK	Acquisition of data; revised the manuscript for intellectual content
David J Werring	Stroke Research Centre, University College London, Institute of Neurology, London, UK	Design and conceptualized study; Interpreted the data; revised the manuscript for intellectual content
Henry Houlden	Neurogenetics Laboratory, The National Hospital of Neurology and Neurosurgery, London, UK	Design and conceptualized study; Interpreted the data; revised the manuscript for intellectual content

APPENDIX 2: CO-INVESTIGATORS

Name	Location	Role	Contribution
Gareth Roberts	Royal Preston Hospital	Site Investigator	Recruited patients
Timothy Jones	St George's Hospital, London	Site Investigator	Recruited patients
Giles Critchley	Hurstwood Park Neurological Centre	Site Investigator	Recruited patients
Pankaj Sharma	Imperial Healthcare, Charing Cross Hospital, London	Site Investigator	Recruited patients
Richard Nelson	Frenchay Hospital, Bristol	Site Investigator	Recruited patients
Peter Whitfield	Derriford Hospital, Plymouth	Site Investigator	Recruited patients
Stuart Ross	Leeds General Infirmary	Site Investigator	Recruited patients
Hiren Patel	Salford Royal Hospital	Site Investigator	Recruited patients
Paul Eldridge	The Walton Centre, Liverpool	Site Investigator	Recruited patients
Kari Saastamoinen	The Royal London Hospital	Site Investigator	Recruited patients
Umang Patel	Sheffield Teaching Hospitals NHS Trust	Site Investigator	Recruited patients
Enas Lawrance	Mayday Hospital, Croydon	Site Investigator	Recruited patients
Subha Vandabona	Mount Gould Hospital, Plymouth	Site Investigator	Recruited patients
David Mendelow	Newcastle University Hopsitals NHS Trust	Site Investigator	Recruited patients
Rachel Teal	Oxford Radcliffe Infirmary	Site Investigator	Recruited patients

REFERENCES

1. Vlak MH, Algra A, Brandenburg R, Rinkel GJ. Prevalence of unruptured intracranial aneurysms, with emphasis on sex, age, comorbidity, country, and time period: a systematic review and meta-analysis. The Lancet Neurology 2011;10:626-636.
2. Johnston SC, Selvin S, Gress DR. The burden, trends, and demographics of mortality from subarachnoid hemorrhage. Neurology 1998;50:1413-1418.
3. Suarez JI, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. The New England journal of medicine 2006;354:387-396.
4. Kim ST, Brinjikji W, Lanzino G, Kallmes DF. Neurovascular manifestations of connective-tissue diseases: A review. Interv Neuroradiol 2016;22:624-637.
5. Flahault A, Trystram D, Nataf F, et al. Screening for intracranial aneurysms in autosomal dominant polycystic kidney disease is cost-effective. Kidney Int 2018;93:716-726.
6. Alg VS, Sofat R, Houlden H, Werring DJ. Genetic risk factors for intracranial aneurysms: a meta-analysis in more than 116,000 individuals. Neurology 2013;80:2154-2165.
7. Bourcier R, Redon R, Desal H. Genetic investigations on intracranial aneurysm: update and perspectives. J Neuroradiol 2015;42:67-71.
8. Huttunen T, von und zu Fraunberg M, Frosen J, et al. Saccular intracranial aneurysm disease: distribution of site, size, and age suggests different etiologies for aneurysm formation and rupture in 316 familial and 1454 sporadic eastern Finnish patients. Neurosurgery 2010;66:631-638; discussion 638.
9. Oike Y, Ito Y, Maekawa H, et al. Angiopoietin-related growth factor (AGF) promotes angiogenesis. Blood 2004;103:3760-3765.
10. Camenisch G, Pisabarro MT, Sherman D, et al. ANGPTL3 stimulates endothelial cell adhesion and migration via integrin alpha vbeta 3 and induces blood vessel formation in vivo. The Journal of biological chemistry 2002;277:17281-17290.
11. Le Jan S, Amy C, Cazes A, et al. Angiopoietin-like 4 is a proangiogenic factor produced during ischemia and in conventional renal cell carcinoma. The American journal of pathology 2003;162:1521-1528.
12. Santulli G. Angiopoietin-like proteins: a comprehensive look. Front Endocrinol (Lausanne) 2014;5:4.
13. Bourcier R, Le Scouarnec S, Bonnaud S, et al. Rare Coding Variants in ANGPTL6 Are Associated with Familial Forms of Intracranial Aneurysm. American journal of human genetics 2018;102:133-141.
14. Hostettler IC, Alg VS, Shahi N, et al. Characteristics of Unruptured Compared to Ruptured Intracranial Aneurysms: A Multicenter Case-Control Study. Neurosurgery 2017.
15. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-291.
16. Hato T, Tabata M, Oike Y. The role of angiopoietin-like proteins in angiogenesis and metabolism. Trends Cardiovasc Med 2008;18:6-14.
17. Hendricks AE, Bochukova EG, Marenne G, et al. Rare Variant Analysis of Human and Rodent Obesity Genes in Individuals with Severe Childhood Obesity. Sci Rep 2017;7:4394.
18. Burkhard P, Stetefeld J, Strelkov SV. Coiled coils: a highly versatile protein folding motif. Trends Cell Biol 2001;11:82-88.
19. Ono M, Shimizugawa T, Shimamura M, et al. Protein region important for regulation of lipid metabolism in angiopoietin-like 3 (ANGPTL3): ANGPTL3 is cleaved and activated in vivo. The Journal of biological chemistry 2003;278:41804-41809.

20. Shimamura M, Matsuda M, Yasumo H, et al. Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase. Arteriosclerosis, thrombosis, and vascular biology 2007;27:366-372.
21. Wang Y, Lam KS, Lam JB, et al. Overexpression of angiopoietin-like protein 4 alters mitochondria activities and modulates methionine metabolic cycle in the liver tissues of db/db diabetic mice. Mol Endocrinol 2007;21:972-986.
22. Oike Y, Akao M, Yasunaga K, et al. Angiopoietin-related growth factor antagonizes obesity and insulin resistance. Nat Med 2005;11:400-408.
23. Yan J, Hitomi T, Takenaka K, et al. Genetic study of intracranial aneurysms. Stroke; a journal of cerebral circulation 2015;46:620-626.
24. Zhang J, Claterbuck RE. Molecular genetics of human intracranial aneurysms. International journal of stroke : official journal of the International Stroke Society 2008;3:272-287.
25. PCNT point mutations and familial intracranial aneurysms. Neurology 2019;93:231.
26. Hamilton A, Tetreault M, Dyment DA, et al. Concordance between whole-exome sequencing and clinical Sanger sequencing: implications for patient care. Mol Genet Genomic Med 2016;4:504-512.
27. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 2015;10:845-858.
28. Kollman JM, Pandi L, Sawaya MR, Riley M, Doolittle RF. Crystal structure of human fibrinogen. Biochemistry 2009;48:3877-3886.

TABLES

Table 1. Baseline characteristics and risk factors

Table 2. Rare non-synonymous variants in the ANGPTL6 gene in patients with familial IA

FIGURE TITLES AND LEGENDS

Figure 1. Patients and controls selection flow diagram

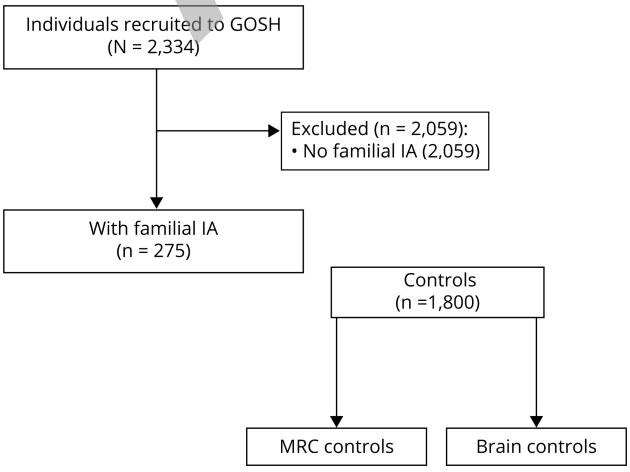

Figure 2. Family trees of individuals with rare genetic variants

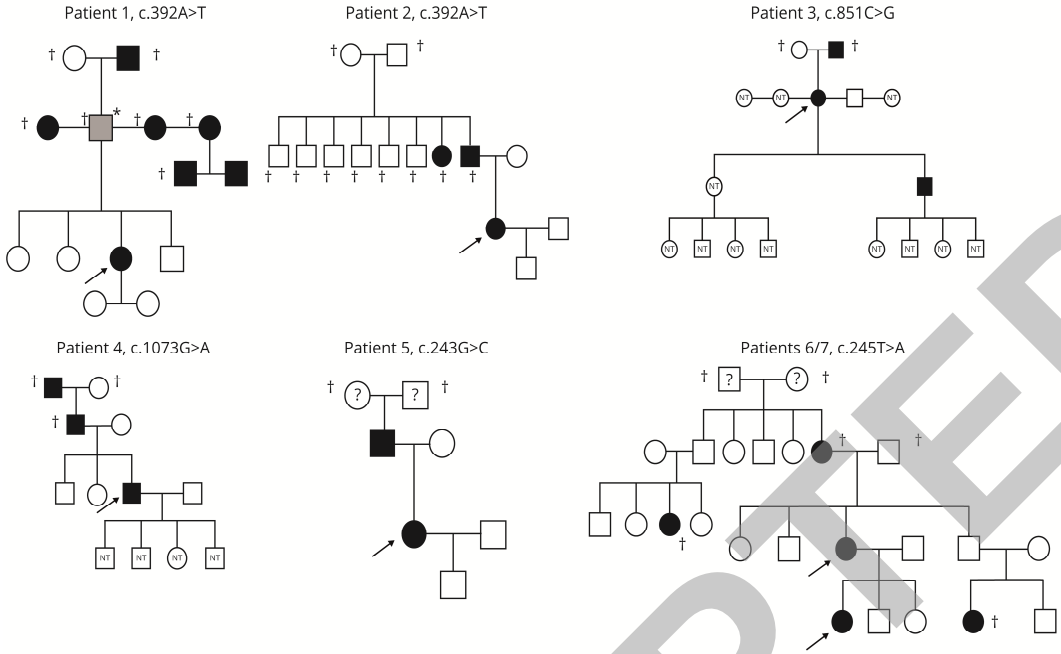

Figure 3. Familial IA with ANGPTL6 genetic variants had typical history and imaging of IA.
Patient 1: 48-year-old woman presenting with subarachnoid haemorrhage within the right sylvian fissure and suprasellar cistern on the axial non-contrast CT examination (a), from a large right terminal M1 segment right middle cerebral artery aneurysm demonstrated on a maximal intensity projection axial CT angiogram (b) and an oblique view of a selective injection into the right internal carotid artery on digital subtraction cerebral angiography (c).
Patient 2: 41-year-old man presenting with subarachnoid haemorrhage within the right sylvian fissure, anterior interhemispheric fissure and right quadrigeminal cistern on the axial non-contrast CT examination (d), from an A1-A2 junction aneurysm of the left anterior cerebral artery demonstrated on axial CT angiography (e) and a Towne's projection from the selective injection of the left internal carotid artery on digital subtraction cerebral angiography (f).
Patient 3: 57-year-old woman presenting with extensive subarachnoid haemorrhage within the sylvian fissures, anterior interhemispheric fissure as well as the perimesencephalic and ambient cisterns on the axial non-contrast CT examination (g), from a posterior communicating segment aneurysm of the left internal carotid artery demonstrated on the lateral projection from the selective injection of the left internal carotid artery on digital subtraction cerebral angiography pre-(h) and post- (i) coil embolisation with satisfactory exclusion from the arterial circulation.
NT=not tested for IA, blacked out = harbouring IA, cross=dead, arrow= individual tested in this study.

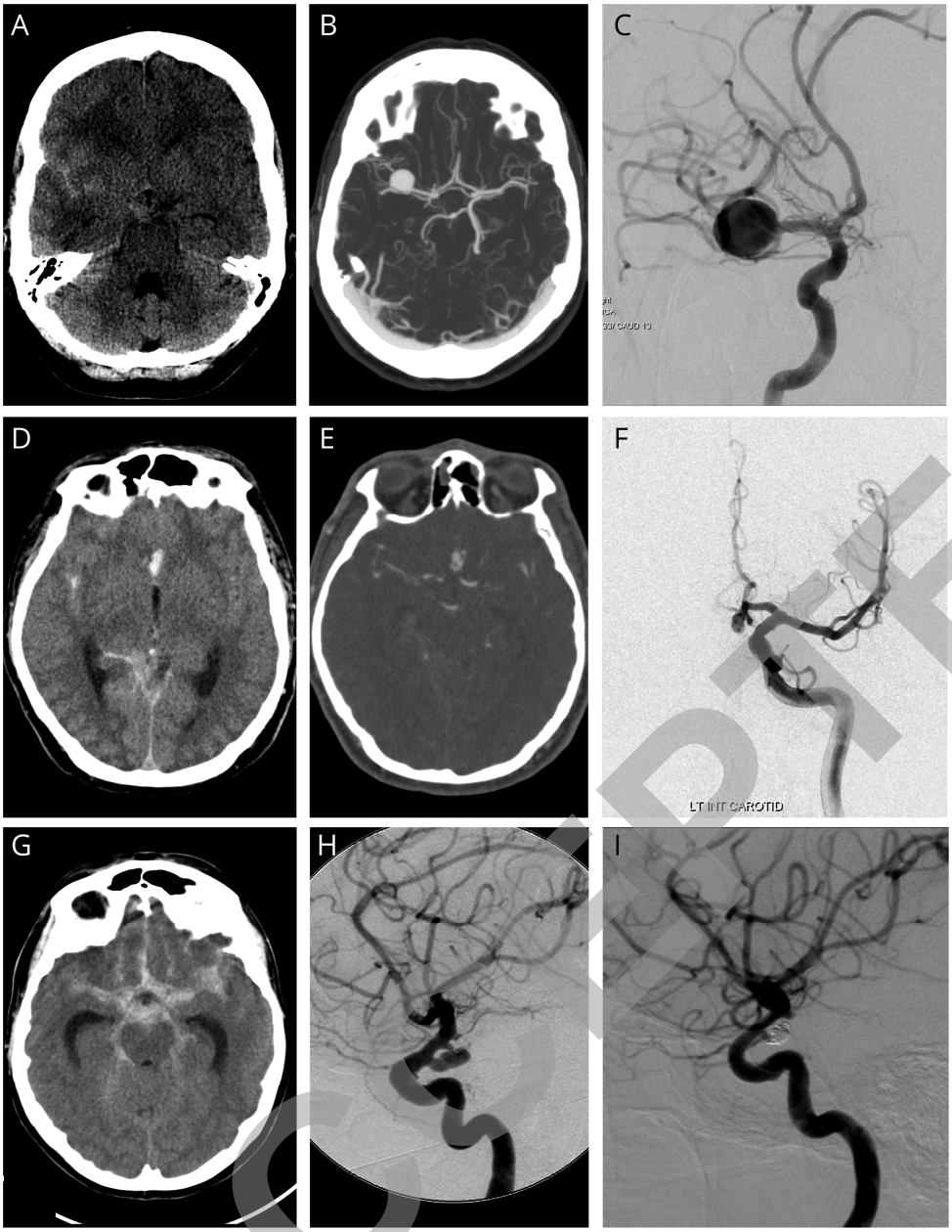

Figure 4. Multiple alignment showing moderate conservation across species and ANGPTL6 homolog of the residues affected by the variants identified in our cohort (variants highlighted in yellow).

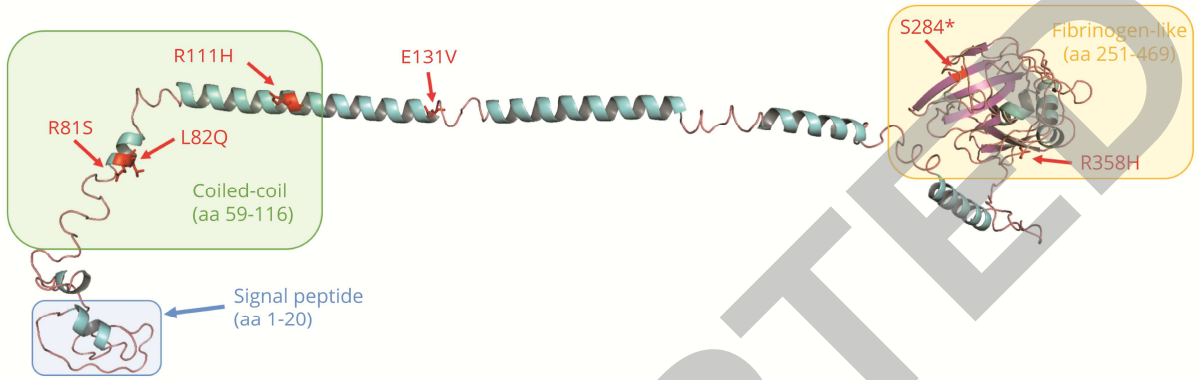

Figure 5. Homology model of human ANGPTL6 protein and respective location of the rare variants identified in this study. ANGPLTL6 homology model created using Phyre2[27] and human fibrinogen beta chain (PDB ID: 3GHG) as the structural template[28]. Three recognisable domains: signal peptide (blue), coiled-coil domain (green) and fibrinogen-like domain (FLD, yellow).

TABLES

Table 1. Baseline characteristics and risk factors

	Familial aneurysms, n=275	Nonfamilial aneurysms, n=2059	p	UIA n=90	aSAH, n=185	p
Age, mean (SD)	51.8 (12.6)	54.5 (12.7)	0.001	53.6 (10.9)	51 (13.3)	0.1
Female Sex	217 (78.9)	1423 (69.1)	0.001	75 (83.3)	142 (76.8)	0.21
Current Smoker, n (%)	108 (39.3)	885 (43)	0.24	26 (28.9)	82 (44.3)	0.02
Current Drinker, n (%)	176 (64)	1384 (67.2)	0.29	52 (58.8)	124 (67)	0.14
PMH						
• HTN, n (%)	98 (35.6)	725 (35.2)	0.89	38 (42.2)	60 (32.4)	0.11
• Hypercholesterolaemia, n (%)	60 (21.8)	508 (24.7)	0.3	33 (36.7)	27 (14.6)	<0.001
• DM, n (%)	6 (2.2)	102 (5)	0.05	2 (2.2)	4 (2.2)	0.97
• OAC, n (%)	22 (8)	96 (4.7)	0.02	4 (4.44)	18 (9.7)	0.14
• Antiplatelets, n (%)	33 (12)	271 (13.2)	0.59	19 (21.19)	14 (7.6)	0.002
• Statins, n (%)	48 (17.5)	417 (20.3)	0.28	26 (28.9)	22 (11.9)	0.001
• Anti HTN, n (%)	81 (29.5)	599 (29.1)	0.9	32 (35.6)	49 (26.5)	0.12
• Previous SAH, n(%)	4 (1.5)	36 (1.8)	0.73	0	4 (2.2)	omit

antiHTN = antihypertensive medication, aSAH = aneurysmal subarachnoid haemorrhage; DM = diabetes mellitus; FU = follow-up; HTN = hypertension; n = number; OAC = oral anticoagulation, PMH = past medical history; SAH = subarachnoid haemorrhage; SD = standard deviation; UIA = unruptured intracranial aneurysm

Table 2. Rare non-synonymous variants in the ANGPTL6 gene in patients with familial IA

AF = allele frequency; Ex = Exon; ExAC = Exome Aggregation Consortium; Het = Heterozygous; Hom = Homozygous; MAF = minor allele frequency; NP = Not present; SIFT = Sorting Intolerant From Tolerant; syn = synonymous
Bold: present in controls as well

Nucleotide change	Exon	Protein Consequence	dbSNP	Het	Hom	MAF (n=500)	ExAC MAF	GnomAD MAF	SIFT	PolyPhen
c.243G>C	Ex2	p.81R>S	rs1166038416	1	0	0.0018	NP	0.00011	Tolerated (0.74)	Benign (0.025)
c.245T>A	Ex2	p.82L>Q	rs979714863	3	0	0.0055	NP	0.000068	Tolerated (0.25)	Benign (0.003)
c.332G>A	Ex2	p.111R>H	rs1310828976	1	0	0.0018	NP	NP	Deleterious (0.04)	Benign (0.254)
c.392A>T[13]	Ex2	p.131E>V	rs576667683	2	0	0.0036	NP	0.0015	Deleterious (0)	possibly damaging (0.724)
c.851C>G	Ex4	p.284S>*	rs201622589	1	0	0.0018	0.0004	0.00045		
c.1073G>A	Ex5	p.358R>H	rs779399477	1	0	0.0018	0.000025	0.000031	Tolerated (0.33)	Benign (0.013)

ANGPTL6 genetic variants are an underlying cause of familial intracranial aneurysms

Isabel C Hostettler, Benjamin O'Callaghan, Enrico Bugiardini, et al.

Neurology published online October 26, 2020
DOI 10.1212/WNL.0000000000011125