Missense mutations in COL4A5 or COL4A6 genes may cause cerebrovascular fibromuscular dysplasia

Case report and literature review

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Abstract

Introduction: Fibromuscular dysplasia (FMD) is a rare and controversial disease that is seldom associated with genes. Here, we report the discovery of 2 missense mutations in COL4A5 and COL4A6 that may be risk factors for causing cerebrovascular FMD. We performed high-throughput sequencing on a patient with FMD and her probable healthy daughter, then annotated the frequency of a variant in a control or general population and assessed its deleterious effects according to published guidelines.

Conclusions: We identified missense mutations in COL4A5 (exon43:c.C3940 > T:p.P1314S) and COL4A6 (exon36:c.C3538 > T:p.P1180S) from the proband and her daughter. Sanger sequencing revealed that these probable causal variants were passed to her from her mother. The two missense mutations may have complex functional effects on the integrity of the cerebral vessel walls, including modulating collagens and promoting angiogenesis expression, may be responsible for cerebrovascular FMD.

Abbreviations: ATS = Alport syndrome, BM = basement membrane, CAD = cerebral artery dissection, CTA = computed tomography angiography, DSA = digital subtraction angiography, FMD = fibromuscular dysplasia, ICA = internal carotid artery, MRI = magnetic resonance imaging, NC1 = non-collagenous, TIA = transient ischemic attack.

Keywords: cerebrovascular, COL4A5, COL4A6, fibromuscular dysplasia

1. Introduction

Fibromuscular dysplasia (FMD) is a rare nonatherosclerotic noninflammatory vascular disease that predominantly occurs in the renal and cerebrovascular regions. It has a striking postpubertal female preponderance of 9:1, of which 7% to 10% cases are hereditary. Though the cause remains idiopathic, genetic as well as environment factors are believed to exist. Ultrastructural characteristics showed the conversion of smooth muscle cells to myofibroblasts that result in arterial stenosis, aneurysm, dissection, or occlusion, which can occasionally lead to transient ischemic attack (TIA), or stroke. In such cases, appropriate ancillary methods may be necessary, especially cerebral angiography could display the typical “string of bead” appearance. It is important to pay attention to this disease since it is an important etiologic agent of stroke among young adults.

Previous studies indicated that the possible genetic markers of FMD include YY1AP1 (chromosome 1q22), COL3A1 (chromosome 2q32.2), NF1 (chromosome 17q11.2), or PHACTR1 (chromosome 6p24.1), which may increase the risk of rupture of the arterial wall. However, the certain causative gene have not been identified. After the diagnosis of cerebrovascular FMD was established, we performed genetic testing and detected the likely pathogenic missense mutations in COL4A5/COL4A6 (chromosome Xq22.3) other than the probable genetic markers described above.

It has been demonstrated the mutations of head-to-head COL4A5/COL4A6 genes which co-encode the components of basement membrane (BM), including smooth muscle, is associate with Alport syndrome (ATS), while ATS associate with FMD. Here we present one clinically suspected case of FMD with the mutations of COL4A5/COL4A6, but have not had the phenotypes of ATS.

2. Materials and methods

2.1. Study subjects and ethics statement

The patient with clinically unambiguous cerebrovascular FMD and 3 other consanguineous individuals including the patient’s daughter were admitted to the Henan Provincial People’s Hospital between January and October 2016. This study was authorized by the ethical committees of the People’s Hospital of Zhengzhou University (2017.NO.46). Written informed consent
was obtained from all patients or their legal surrogates for using the clinical data and blood samples.

2.2. DNA preparation and screening for pathogenic genes

The patient and her daughter’s genomic DNA were isolated from peripheral blood using the QIAquick PCR purification kit. A total of ≥0.5 μg DNA per sample was used for library generation. The targeted sequences were captured from genomic DNA using the SureSelect Human All ExonV6 kit (Agilent) and sequenced on Illumina Hiseq XT for paired-end 150 bp reads. Valid sequencing data were mapped to the reference genome (GRCh37/hg19) by BWA software (Li et al 2009-1) to obtain the original mapping result in BAM format. Subsequently, Samtools (Li et al 2009-2) and Picard (http://broadinstitute.github.io/picard) were utilized to sort bam files, and mark duplicate reads, respectively. Then, statistical analysis was performed to compare the sequencing depth and coverage. In the present study, all SNP/InDel were obtained by Samtools and filtered according to the international standard of filtration. Additionally, SNP/InDel was annotated using ANNOVAR (WangYK et al.). Finally, deleterious mutations were classified according to the ACMG standard and guidelines.[11]

2.3. Polymerase chain reaction (PCR) analysis

The variants were confirmed by bidirectional Sanger DNA sequencing using primers (COLA45: forward 5'-TGCGCACCT- CAGTTAGCCAT-3’ and reverse 5’- AGCTGATCCAGG- TACTCCAC -3’; COL4A6: forward 5’-CTTCAGTACTCTGC CTCTGG-3’, and reverse 5’-GCCTCAGCTAGC CTCTACA-3’). Sanger sequencing was performed by Sangon Biotech (Shanghai, China) to validate the sequencing results.

3. Results

3.1. Clinical features

The proband was a 40-year-old woman who was previously healthy with no identifiable risk factors for stroke and presented with recurrent mild attacks resembling TIA. Ten days before the onset of symptoms, she participated in a group dance and showed moderate vigorous physical activity, such as constant head nodding, and rapid head movements. Clinical manifestations included vertigo and right hemiplegia, progressing to secondary right central facial palsy and aphasia, lasting about 5 to 20 minutes, 5 and 6 times per day, over a 6-day period. A complete physical examination revealed slight central facial palsy on the right side, severe weakness in the right upper extremities (3/5; MRC scale), slightly better strength in the right lower limb (4/5; MRC scale), activity reflexes in the right limbs (3/4; NINDS scale), and positive pyramidal tract signs on the right. Magnetic resonance imaging (MRI) (Fig. 1) was sequentially performed in the proband, which showed suspicious hyperintense mural hematoma with eccentric superior lumen of left vertebral artery, pontine infarction, and double lumen sign in

Figure 1. Imaging features over time in the following order: A–B, E–F–G–H, C–D. A: Suspicious hyperintense mural hematoma with eccentric superior lumen of left vertebral artery on T1-weighted sequence, shown on (B), magnified image of A, with arrow. C: Ischemic stroke in the pontine on T2-weighted MRI, and slender right vertebral artery, shown on (F). G: The typical "string of bead" stenosis in the left V2-3 vertebral artery, shown on (H), magnified image of G, with arrowhead.
basilar artery. Digital subtraction angiography (DSA) (Fig. 1) showed the typical “string of bead” appearance in the left vertebral artery of C2–C3. Special screening for events related to neurological injury, including cerebrovascular risk factors, coagulopathy factors, and inflammatory component, were unremarkable.

The patient was admitted to the hospital within 24 hours of suspected acute cerebral infarction onset. She was given clopidogrel 75 mg daily, aspirin 100 mg daily, and low molecular heparin 6000 u twice daily. Her presenting symptoms of TIA were relieved with this treatment, and the diagnosis of FMD was suggested.

3.2. Follow-up information

Interestingly, computerized tomography angiography (CTA) at follow-up revealed a dissecting aneurysm in the right internal carotid artery (ICA) and stenosis in the right, but with no compensatory collateral vessels after the neurological signs had completely returned to normal in 18 days (Fig. 2).

Carotid ultrasound examination then revealed a high-grade stenosis with no plaque of the right C2 ICA in 19 days. CTA showed compensatory collateral vessels that were similar to those observed in Moyamoya disease after 3 months. No aneurysms were observed through CTA of abdominal aorta during the follow-up (Fig. 3).

Thus, the diagnosis of clinically unambiguous cerebrovascular FMD was made. Given its association with genes, we performed high-throughput sequencing for the patient, her daughter and her parents, which were also analyzed by Sanger sequencing.

3.3. Mutation detection and polymorphisms

High-throughput sequencing was performed for the proband and her daughter as an unbiased approach to identify the causal mutation in the family (Fig. 4). Sanger sequencing was also performed to validate the high-throughput sequencing results of COL4A5/COL4A6 variants in the probable familial FMD cases.

Target region capture sequencing identified 208 potentially deleterious variants in the proband that met the filtering criteria, and the mutations included COL4A5 (exon 43: c.C3940 > T: p.P1314S) and COL4A6 (exon 36: c.C3538 > T: p.P1180S), which were located on Xq22.3. This variant was a strong candidate because COL4A5/COL4A6 encodes collagen IV, which is a constituent of α5β56 that exists in the BM of smooth muscles. [9]

The proband’s mother and her daughter do not have the disease, but Sanger sequencing revealed that they also carried these variants.
These mutations have no genomic SuperDups and Repeat annotations. The frequency in 1000 genomes, ExAC were <0.01, and have not been reported in avsnp, clinvar, GWAS, and esp6500. This demonstrated that the variants identified in our study are rare polymorphisms.

Deleterious effects were evaluated using computational and predictive data. Multiple lines of computational (MutationTaster, LRT, and FATHMM) evidence supported a deleterious effect of the c.C3940T nucleotide variant in COL4A5, but only one (FATHMM) showed that c.C3538T is deleterious.

The c.C3940T nucleotide variant in COL4A5 has been reported in ATS,[12] while COL4A6 may be associated with deafness,[13] the likely pathogenic (COL4A5) and uncertain significance (COL4A6) are defined according to ACMG guideline.[11] However, more recent studies have reported that the collagen gene, especially COL4A1/COL4A2, some of which are not located in non-collagenous (NC1) domain (Uniprot), are related with angiopathy.[14] However, there was no evidence of correlation between collagen gene and FMD.

Therefore, although none of the amino acid changes were predicted in the structural domains, it is reasonable to suspect that the mutations were pathogenic.

4. Discussion

Cerebral artery dissection (CAD) was diagnosed in the proband at the age of 40 years after adiological results suggested infarction, stenosis, aneurysm, and dissection near the level of C1-C2. A recent date demonstrated that CAD is the second leading cause in young stroke patients with high incidence of 8% to 25%.[15,16] Data suggest that trauma, hypertension and FMD are the potential risk factors.[17] The appearance of the typical “string of bead” indicated the diagnosis FMD, which mainly affect the medial fibroplasia.[15] Population-based studies have documented that females are more affected than males, and there

![Collagen Gene Analysis](image1)

**Figure 4.** COL4A5/COL4A6 mutations in a probable cerebrovascular-FMD family. A. The information of high-throughput sequencing of our proband (subject II.2, with arrow) and her daughter (subject III.2) shown below the pedigree symbols. Analysis of other family members was also performed by medical history and Sanger sequencing. No neurological findings were detected in the other family members, but the two mutations were also detected in her mother (subject I2). The subject III and subject II4 were not sequenced (EU). B. The result of Sanger sequencing shown on B.
may have familial clustering (suspected incomplete dominant). Therefore, it is reasonable to consider diagnosis of FMD in stroke patients with dissection who were previously healthy, and a genetic test appears desirable.

The possible disease-associated mutations in COL4A5 (c. C3940T;p.P1314S) and COL4A6 (c.C3338T;p. P1180S) were detected identical by descent from the subjects I2, I12 and I18, which indicated that they may inherited as an X-linked incomplete dominant trait. This suggest that COL4A5/COL4A6 genes may not be ATS-specific and may contributing to FMD, which support the common variant/multiple disease hypothesis.[20] This head-to-head COL4A5/COL4A6 genes are located on Xq22.3 and use a common promotor. Collagen, type IV, alpha 5, and alpha 6 form heterotrimers and are abundant components of BM, including those of the smooth muscle.[19] The 2 mutations exist in the triple-helical region (Uniprot), other than NC1 domain that may affect the stability of BM. The P1314S mutation has been reported to associate with ATS.[11] However isolated cases of FMD mimicking ATS have been reported, which is caused by mutation in collagen genes.[21] Accordingly, we hypothesized that these missense mutations in COL4A5/ COL4A6 may lead to intracellular deposition of α3 or α6 chain, followed by a change in the α5α5α6 chain structure, and are responsible for lowering BM strength and integrity. The possible reduced stress of BM was perhaps accompanied by its damage. The nuclear changes led to reprogramming of cellular proliferation, adhesion, migration, gene expression, and differentiation, including smooth muscle cells. Cumulative structural changes in tissue may have developed after prolonged inappropriate stress, which caused the conversion of smooth muscle cells to myofibroblasts that resulted in arterial stiffness, aneurysm, dissection, or occlusion. Finally, biomechanical factors induced morphological alterations of SM, resulting in the formation of typical “string of bead” vessels. The advanced compensatory collateral vessels similar to those observed in Moyamoya disease may have resulted due to deletion of the paired COL4A6, which is involved in the regulation of angiogenesis.[22]

Nonetheless, the findings have not been scientifically assessed. First, a systematic examination, including laboratory and radiological studies of other members of the family was not conducted. Second, there is no direct evidence of vascular pathology and animal trials.

In conclusion, whether the mutations of the paired COL4A5/ COL4A6 genes can lead to abnormalities in basement membrane structure and the development of cerebrovascular-FMD needs further study. Finding disease-predisposition genes may help to identify patients that are at risk for recurrent stroke, and guide decisions regarding therapeutic strategies.

Acknowledgments
The authors would like to thank the families for their participation in this study. The authors also thank Yingying Shi for helping with clinical and genetic analysis in this project.

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