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COL4A1 and COL4A2 Mutations Analyses with Perinatal Arterial İschemic Stroke

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Abstract: Perinatal arterial ischemic stroke (PAIS) is one of the frequent causes of mortality and morbidity, but its etiology remains unclear. *COL4A1* and *COL4A2* mutations are monogenetic causes of weakness of the basement vascular membranes resulting in cerebral small-vessel disease, cerebral hemorrhage, and porencephaly. We hypothesized that variations in the *COL4A1* and *COL4A2* genes cause PAIS and performed mutation screening of these genes in 17 PAIS patients by whole-exome sequencing. Clinical, demographic, and laboratory data of the 17 PAIS patients were obtained by evaluating hospital files retrospectively. Patients included in the study were invited to the clinic for *COL4A1* and *COL4A2* mutation analysis. Results: The patient group consisted of 13 females (76.5%) and four males (23.5%) with a mean age of 107.4 ± 11.5 months. Maternal/fetal and prothrombotic risk factors identified in 52.9% and 94.1% of the patients, respectively. Whole-exome sequencing analysis did not reveal *COL4A1* and *COL4A2* pathological mutations in any of the patients. Although we did not find an association between PAIS and *COL4A1* and *COL4A2* variations, we believe that new studies with larger patient populations may reveal such a relationship.
Keywords: *COL4A1*; *COL4A2*; perinatal stroke; congenital hemiplegia; cerebral palsy

INTRODUCTION

Perinatal ischemic stroke is one of the frequent causes of morbidity and severe long-term neurologic and cognitive deficits, including cerebral palsy, epilepsy, neurodevelopmental disabilities, behavioral disorders, and impaired vision and language function[1]. It defined as “a group of heterogeneous conditions involving focal disruption of cerebral blood flow secondary to arterial or venous thrombosis or embolization between the 20th week of fetal life through the 28th postnatal day”, and the diagnosis should always be confirmed by neuroimaging or by neuropathological investigations[2]. The two main categories are periventricular arterial ischemic stroke (PAIS) and cerebral sinovenous thrombosis. The incidence of PAIS ranges from 1 in 2300 to 5000 births[3,4]. The etiology of PAIS has not been fully established but considered to be the result of multifactorial risk factors during pregnancy and delivery[5-8].

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The basement membrane (BM), including the vascular BM, is an extracellular matrix associated with overlying cells that is important for proper tissue development, stability, and physiology[9]. Type IV collagen, which is a significant protein expressed in many tissues, including the vascular endothelia, is critical for the formation of stable BMs during embryonic development[10,11]. α1(IV) and α6(IV), encoded by *COL4A1* to *COL4A6*, respectively, are considered to be the classical type IV collagen alpha chains[12]. Dominant missense mutations in *COL4A1* and *COL4A2* are associated with multisystemic disorders, including intracerebral hemorrhage, porencephaly, nephropathy, ocular malformation, and myopathy[13,14]. Van der Knaap et al.[15]. reported focal disruptions and a significant increase in the thickness of the vascular BM of human skin capillaries due to *COL4A1* and *COL4A2* mutations. It has suggested that focal disturbances of the vascular BM can predispose to bleeding.

In contrast, the swelling of vascular endothelial cells and the increased thickness of the BM can lead to narrowing of vessels and thus predispose to ischemic damage. Previous studies are generally about perinatal hemorrhage and multisystemic involvement of *COL4A1* and *COL4A2* mutations. Our primary aim was to identify possible relations with *COL4A1* and *COL4A2* mutations with PAIS.

We hypothesized that *COL4A1* and *COL4A2* mutations cause PAIS, and herein, we report on the study of 17 PAIS patients who examined for *COL4A1* and *COL4A2* mutations by whole-exome sequencing.

MATERIALS AND METHODS

Seventeen patients diagnosed with PAIS and followed-up at the Eskisehir Osmangazi University Hospital, Department of Pediatric Neurology, between January 2011 and September 2016 were the participants of this study. The Clinical Research Ethics Committee approved the study protocol of the Eskisehir Osmangazi University School of Medicine, and the study conducted according to the Declaration of Helsinki. I have written informed consent obtained from the parents of each patient. Clinical, demographic, neuroimaging (cranial magnetic resonance imaging (MRI), and laboratory data of the PAIS patients were obtained by evaluating hospital files retrospectively.

The diagnosis of PAIS based on clinical features, neurological examination, and cranial MRI findings. Inclusion criteria were PAIS confirmed using MRI and a follow-up period of more than six months. Patients who had a congenital cerebral anomaly, cerebrovascular disorder, brain tumor, sequel of hypoxic-ischemic encephalopathy, cortical dysplasia, central nervous system infection, preterm birth, or trauma excluded.

All patients' prenatal and natal history, as well as maternal risk factors, were obtained using a standard form. Prothrombotic risk factors such as factor V Leiden, methylenetetrahydrofolate reductase (*MTHFR*) (C677T and A1298C), and prothrombin *G20210A* mutations; homocysteine, protein C/S, lipoprotein (a), antithrombin III (ATIII), and factor VIII levels; and the presence of anticardiolipin antibodies and lupus anticoagulant obtained from reviews of the medical histories.

Whole-exome sequencing for *COL4A1* and *COL4A2* mutations

Patients included in the study were invited to the clinic for *COL4A1* and *COL4A2* mutation analysis. Written informed consent obtained from both parents. Genomic DNA from the patients captured using the SureSelectXT Human All Exon v5

Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced with six samples per lane on an Illumina HiSeq 2000 (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. Image analysis and base calling performed by sequence control software real-time analysis and CASAVA software v1.8 (Illumina). Exome data processing, variant calling, and variant annotation performed as previously described[16-19].

RESULT AND DISCUSSION

A total of 22 patients were excluded because of hypoxic-ischemic encephalopathy (10), periventricular leukomalacia (7), malformation (3), and metabolic disorders (2), leaving a study population of 17 children.

The patient group consisted of 13 females (76.5%) and four males (23.5%) with a mean age of 107.4 ± 11.5 months. The average duration of follow-up was 46 months (range: 9–122). None of the patients had a positive family history of stroke. All patients had delivered by cesarean section, and there were no reported placental abnormalities. All births occurred at term, and the average birth weight was 3100g (1900–4700g). Six parents were involved in consanguineous marriages. All of the patients' echocardiography results were normal. Maternal/fetal risk factors were associated with 52.9% of patients (intrauterine growth retardation (IUGR) (35.3%), twin pregnancy (5.9%), and abnormal vaginal bleeding (11.7%)). Epilepsy (70.6%), mild/severe intellectual disability (64.7%), behavioral disorders (17.6%), unilateral spastic cerebral palsy (100%), and congenital hemiplegia (100%: 10 right and seven left) found in the patients.

Prothrombotic risk factors detected in 94.1% of the patients. *MTHFR* mutations detected in 76.5% of the patients, with the A1298C heterozygous carrier state present in 7 patients, the A1298C homozygous carrier state in 3, the C677T heterozygous carrier state in 2, the C677T homozygous carrier state in 2, and the combined (C677T+A1298C) mutation carrier state in 1. Factor V Leiden heterozygous mutation found in 1 patient. Combined *MTHFR* and factor V Leiden mutations found in 2 patients. In all patients, lupus anticoagulant was negative, and factor VIII levels were within normal limits. Prothrombin G20210A mutation increased levels of anticardiolipin antibodies, protein S deficiency, hyperhomocysteinemia, protein C deficiency, and antithrombin III deficiency not detected in any of the patients. We conducted a whole-exome sequencing analysis for *COL4A1* and *COL4A2* mutations in the 17 PAIS patients, which showed no pathological mutations in either gene in any of the patients (Table 1).

PAIS is one of the most typical forms of pediatric stroke and 17 times greater than the incidence of AIS in children[20]. Approximately 60% of perinatal strokes result in neurological deficits, including cerebral palsy, neurocognitive deficits, language impairment, behavioral disorders, and epilepsy, and emerging deficits during the school years such as learning disabilities[21]. In our study, all 17 patients had congenital hemiplegia (unilateral spastic cerebral palsy), 70.6% had epilepsy, 64.7% had an intellectual disability, and 17.6% had behavioral disorders. However, the pathophysiology and risk factors of PAIS remain mostly unclear. Maternal risk factors include infertility, preeclampsia, chorioamnionitis, placental vasculopathy, coagulation disorders, and cocaine abuse; fetal risk factors include growth restriction, intrauterine asphyxia, heart diseases, infections, congenital vascular anomalies, dehydration, and

traumatic delivery; and there are also prothrombotic risk factors. However, the role of these factors on the occurrence of PAIS remains unclear[22-24].

In our study, maternal/fetal risk factors were present for 52.9% of patients, including pregnancy with twins, abnormal vaginal bleeding, and IUGR. Stroke more frequently affects the left hemisphere and often involves the middle cerebral artery (MCA) territory[25]. In our study, 10(7) of 17 patients had left MCA stroke.

Table 1: Clinical and Radiological Characteristics and Risk Factors of Patient

Patient no	Sex/ Age (mo)	Side of hempl egia	Clinical features	MRI findings (stroke localization)	Risk factors	COL4A1/ COL4A2 mutation
1	38/M	R	Epilepsy, intellectuel disabilty	L. MCA	MTHFR A1298C Het.+FVL Het.	Negative
2	42/F	L	Intellectuel disabilty	R. MCA	MTHFR A1298C Het.	Negative
3	50/F	L	Intellectuel disabilty	L. MCA	MTHFR A1298C Hom., IUGR	Negative
4	56/M	L	Epilepsy, intellectuel disabilty	R. MCA	MTHFR C677T Hom., IUGR	Negative
5	64/M	R	Intellectuel disabilty	L. MCA	MTHFR A1298C Hom.	Negative
6	72/F	R	Epilepsy	L. MCA	MTHFR A1298C Het.	Negative
7	96/F	R	Epilepsy, intellectuel disabilty	L. MCA	MTHFR A1298C Hom., Twin dizygotic, IUGR	Negative
8	96/F	L	Intellectuel disabilty	R. MCA	MTHFR A1298C Het.	Negative
9	120/F	R	Epilepsy, intellectuel disabilty	L. MCA	FVL Het., IUGR	Negative
10	120/F	R	Epilepsy, intellectuel disabilty	L. MCA	MTHFR A1298C Het.	Negative
11	132/ M	L	Epilepsy, intellectuel disabilty	R. MCA	MTHFR A1298C Het.+FVL Het.	Negative

Patient no	Sex/ Age (mo)	Side of hempl egia	Clinical features	MRI findings (stroke localization)	Risk factors	COL4A1/ COL4A2 mutation
12	132/F	R	Epilepsy	L. MCA	MTHFR C677T Het., abnormal vaginal bleeding	Negative
13	136/F	R	Intellectuel disabilty	L. MCA	MTHFR C677 Hom., IUGR	Negative
14	144/F	R	Epilespy	L. MCA	MTHFR A1298C Het.	Negative
15	166/F	L	Epilepsy	R. MCA	MTHFR C677T Het.,IUGR	Negative
16	175/F	L	Epilepsy, intellectuel disabilty	R. MCA	MTHFR C677T Het.+ MTHFR A1298C Het.	Negative
17	187/F	R	Epilepsy, intellectuel disabilty	L. MCA	Abnormal vaginal bleeding	Negative

Abbreviations: F:female, **M:**male, **mo:**months, **R:** right, **L:**left, **MCA:**middle cerebral artery, **MTHFR:** methylenetetrahydrofolate reductase, **het:**heterozygosity, **hom:**homozygosity, **FVL:** factor V Leiden; **IUGR:** intrauterin growth retardation

COL4A1 and *COL4A2* genes located at chromosome 13q34. They encode the collagen chains a1(IV) and a2(IV), which constitute a significant component of the vascular BM[26], *COL4A1* variants that cause an autosomal dominant disorder affecting the structural integrity of the BM resulting in perinatal cerebral hemorrhages and porencephaly first reported in 2005[27]. In 2012, it stated that mutations in the *COL4A2* gene at the same chromosomal locus caused a similar phenotype of cerebral small-vessel disease (SVD) manifesting as intracerebral hemorrhage (ICH), early-onset porencephaly, and nephropathy[28,29]. In addition to cerebrovascular disease, *COL4A1* and *COL4A2* mutations confirmed to cause ocular, renal, and muscular disorders. Ophthalmologic findings including bilateral tortuosity of the second- and third-order arteries, hemorrhagic lesions, and the Axenfelde Rieger anomaly characterized by microcornea, congenital, or juvenile cataracts, increased intraocular pressure, iris hypoplasia, retinal detachment, and optic nerve excavation[9,14,30,31]. Renal involvement manifests as hematuria and renal cysts[9,30]. Muscle cramps involving a variety of muscles have been reported, with associated persistent elevation of serum creatine kinase concentrations (HANAC syndrome)[31].

Neurological features *COL4A1* and *COL4A2* mutations include porencephaly, SVD, ICH, ischemic strokes, white matter hyperintensity (WMH), dilated perivascular

spaces, intracranial aneurysms, seizures, congenital hemiplegia, myopathy, migraine, cognitive impairment, and dementia[15,26,32,33]. In addition to pre- and perinatal hemorrhages, *COL4A1*, and *COL4A2* mutations also cause sporadic and recurrent ICH in young and old patients. Van der Knaap et al.[15]. reported that *COL4A1* and *COL4A2* mutations might cause an ischemic stroke. Genetic studies about ischemic stroke and hemorrhagic strokes have generally been pursued separately and have focused on different etiological causes. Rannikmäe et al.[34]. conducted a meta-analysis on *COL4A2* mutations associated with ischemic and hemorrhagic stroke and found that the same genetic signal is associated with clinically evident sporadic ischemic and hemorrhagic stroke. Another study found that *COL4A1* and *COL4A2* cause deep ICH and symptomatic cerebral SVD phenotypes, which is suggestive of associations in the same direction for these single nucleotide polymorphisms with other cerebral SVD phenotypes: lacunar ischemic stroke and WMH in ischemic stroke cases[35]. According to these studies, we hypothesized that *COL4A1* and *COL4A2* mutations cause both perinatal hemorrhagic stroke and PAIS.

To the best of our knowledge, this is the first study to attempt to link *COL4A1/COL4A2* mutations and PAIS. However, we could not find an association between them. Our principal study limitation is the small patient population. Although PAIS is a significant cause of morbidity and mortality, its pathophysiology is not fully understood. *COL4A1* and *COL4A2* mutations are still a potential risk factor for PAIS. We believe that new studies with a large patient population may allow us to understand whether there is a relationship between *COL4A1* and *COL4A2* mutations and PAIS.

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CONFLICT OF INTEREST

There were no conflicts of interest with related parties in this study.

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