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Further refinement of *COL4A1* and *COL4A2* related cortical malformations

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ABSTRACT

Mutations in *COL4A1* have been reported in schizencephaly and porencephaly combined with microbleeds or calcifications, often associated with ocular and renal abnormalities, myopathy, elevated creatine kinase levels and haemolytic anaemia. In this study, we aimed to clarify the phenotypic spectrum of *COL4A1/A2* mutations in the context of cortical malformations that include schizencephaly, polymicrogyria and/or heterotopia.

Methods: We screened for *COL4A1/A2* mutations in 9 patients with schizencephaly and/or polymicrogyria suspected to be caused by vascular disruption and leading to a cerebral haemorrhagic ischaemic event. These included 6 cases with asymmetrical or unilateral schizencephaly and/or polymicrogyria and 3 cases with bilateral schizencephaly.

Results: One *de novo* missense *COL4A1* mutation (c.3715 G > A, p.(Gly1239Arg)) and two *COL4A2* mutations were found, respectively in one familial case (c.4129G > A, p.(Gly1377Arg)) and one sporadic patient (c.1776 + 1G > A). In three other cases, *COL4A1* variants of unknown significance were identified. None of our patients demonstrated neuromuscular or hematological anomalies. Brain malformations included a combination of schizencephaly, mainly asymmetrical, with porencephaly or ventriculomegaly (3/3 mutated patients). We did not observe microbleeds or microcalcifications in any of our cases, hence we do not believe that they represent a distinctive feature of *COL4A1/A2* mutations.

Conclusions: Our study further emphasizes the need to search for both *COL4A1* and *COL4A2* mutations in

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children presenting with uni- or bilateral polymicrogyria with schizencephaly, even in the absence of intracranial microbleeds, calcification or associated systemic features.

1. Introduction

Prenatal stroke occurs in many different neurological disorders, both acquired or genetic, and may be diagnosed antenatally or after birth. They encompass a large spectrum of brain lesions depending both on the timing and mechanism of the injury in the developing brain. (Bejar et al., 1990; Govaert, 2009; Larroche et al., 1990, 1994).

Schizencephaly is a rare cortical malformation characterized by the presence of a polymicrogyric cortex lining a cleft extending from the surface of the pia mater to the lateral ventricles. The spectrum of the cleft varies: from a thread of cerebrospinal fluid connecting the subarachnoid spaces to the ventricles which is encircled by abnormal gray matter to a wide communication between the subarachnoid space and the ventricles with edges of abnormal cortex (Barkovich and Kjos, 1992; Packard et al., 1997) (Nabavizadeh et al., 2014). Accumulated evidence supports the fact that hydranencephaly, schizencephaly, porencephaly and polymicrogyria represent a continuum of brain injury depending on the timing and the severity of the insult. For this reason, schizencephaly is also referred to as embryonic or early foetal porencephaly occurring before 24 weeks gestation (Yakovlev and Wadsworth, 1946) (Govaert, 2009). The causes of schizencephaly are heterogeneous and remain poorly understood (Curry et al., 2005).

COL4A1 and *COL4A2* mutations have been reported in relation to a broader spectrum of cerebrovascular, renal, ophthalmological, cardiac, and neuromuscular abnormalities, encompassing "*COL4A1* mutation-related disorders" (Kuo et al., 2012). In addition, recent data suggests that mutations in *COL4A1* gene, a major cause of inherited cerebrovascular disease, might lead to schizencephaly (Meuwissen et al., 2015; Yoneda et al., 2013). However, although the authors emphasized the presence of intracranial calcifications and hemosiderin deposition in all 5 patients reported so far with *COL4A1* related schizencephaly, the distinctive features for *COL4A1* related schizencephaly are still unclear.

To obtain further insights into the clinical and radiological features that should lead to the screening of patients for *COL4A1A/A2* mutations, we tested a cohort of 9 patients with cortical malformations including schizencephaly, polymicrogyria and/or heterotopia. These cortical malformations were suspected to vascular in origin but without any identifiable risk factors.

2. Methods

2.1. Case ascertainment

A total of 9 patients with polymicrogyria, with or without schizencephaly, and heterotopia were investigated. All tested individuals and legal caretakers gave their informed consent for the study, according to the local ethical committee requirements. This is a selected population comprising patients who were either referred to the authors directly or those in whom clinical details and scans were sent to the authors (NBB and MC) for an opinion. Because of the particular interest of two authors (NBB and NB) in cortical malformations, most patients were referred initially in view of suspected developmental cortical malformations.

2.2. Clinical information, brain MRI and classification

All patients were known personally to at least one of the authors who re-examined them for the purpose of the study. Both the clinical information and brain magnetic resonance images (MRIs) were obtained and centralized by NBB at the Paediatric Neurology unit of Necker Enfants Malades Hospital, Paris Descartes University. Detailed information regarding family history, pre and perinatal events, motor development, cognitive function, neurological examination including occipito-frontal head circumference (OFC) and ophthalmological features was recorded. With regards to the epilepsy, age of seizure onset, main seizure type, and response to antiepileptic drugs were recorded.

Brain MR images were reviewed by at least two investigators (NBB and NB) for the purpose of classifying patients. The scans were reviewed using a proforma in which the following abnormalities (using widely accepted criteria) were recorded. Cortical malformations were classified as follows: schizencephaly was defined as previously (Barkovich and Kjos, 1992; Packard et al., 1997) as either a cortical cleft lined with gray matter clearly extending from the pial surface to the ventricle or as a cleft that was closely associated with a focal ventricular abnormality. The schizencephalic cleft was classified morphologically as a closed-lip, if there was gray matter lining the walls of the cleft extending from the cerebral surface to the lateral ventricle, or open-lip, if cerebrospinal fluid could be seen between gray matter-lined walls through the entire length of the cleft. Porencephaly was defined as an area of cystic parenchymal loss communicating with the ventricles and/or the brain surface. Polymicrogyria was diagnosed according to the three recognized criteria (Leventer et al., 2010) (i) irregular surface of cortex; (ii) thickened or overfolded cortex aspect; (iii) irregularity at the gray-white interface. The morphological analysis of heterotopia was performed as proposed previously (Barkovich, 2000).

The presence or absence of the corpus callosum, septum pellucidum, cortical dysplasia and other brain anomalies, in particular white matter changes suggestive of leukoencephalopathy, was additionally noted. Also, any sign of recent or old intracranial haemorrhage, including silent microbleeds and microcalcification were noted.

2.3. Mutation analysis

Blood samples for DNA preparation and genetic investigations were obtained with informed consent from patient's parents. DNA was extracted using standard protocols. For each patient, array CGH was normal and Next Generation Sequencing (NGS) of a panel list of 56 MCD genes known to be associated with cortical malformations was negative (additional table).

For 7/9 patients negative for this panel sequencing, standard PCR amplification and Sanger sequencing of all *COL4A1* and *COL4A2 coding exons* was performed using standardized procedures. The remaining 2 patients underwent whole exome sequencing followed by targeted Sanger sequencing of identified variants. When available, patient's relatives were also sequenced. For cDNA sequencing, total RNA was isolated from EBV-transformed lymphoblastoid cell lines of fibroblasts with Trizol reagent (Invitrogen) and cDNA obtained with *Superscript*™ III reverse transcriptase (Invitrogen). To establish the *de novo* nature of mutations, the proband's DNA and DNA from both non-carrier parents were tested for nine sets of microsatellites using the AmpFlSTR Profiler PCR Amplification Kit (Applied Biosystems). The reaction was carried out according to the manufacturer's instructions, loaded on an ABI 3130 genetic analyzer (Applied Biosystems) and the fragments were analyzed using GeneMapper v4.0 software (Applied Biosystems). In addition, 3 of the 6 patients without any pathogenic point mutation variant and for whom enough DNA was available, were screened for *COL4A1/COL4A2* deletions and duplications by Quantitative Multiplex Short Fragment (QMPSF) analysis using standard procedures.

The frequency of each candidate variant was sought in control

Table 1
Individual data on growth, development and clinical examination of 9 patients with schizencephaly.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
COL4A1 mutation	Absent	Absent	c.3715 G > A, p.(Gly1239Arg) Pathogenic	c.4727C > T, p.(Ser1576Leu)* Uncertain significance	c.1588C > T, p.(Pro530Ser)* Uncertain significance	Absent	c.1085-37T > A* Uncertain significance	Absent	Absent
COL4A2 mutation	c.4129G > A, p.(Gly1377Arg) Pathogenic	Exon 24, c.1776+1G > A, p? Pathogenic	not tested	Absent	Absent	Absent	Absent	Absent	Absent
Sex	Female	Male	Female	Male	Male	Female	Female	Male	Male
Birth (gestational week)	36	39.28571429	39	39	36	38	39	39	36
Birth weight (g)	1740	4000	2910	4040	2920	3050	3220	3100	2375
Birth weight (SD)	−2.16	1.34	−0.71	1.42	0.44	0	−0.04	−0.58	−0.81
Birth Head circumference (cm)	29	NE	34	37	32	31.5	34	34	31
Birth Head circumference (SD)	−3.21	NE	−0.11	2	−0.44	−1.46	−0.11	−0.35	−1.12
Mean age at investigation (y)	4	11	5	0.58	9.5	5	5.5	8.5	6
Growth parameters									
Head circumference (cm)	48.5	51	46	48		46	48	54	46
SD	−2	−2	−4.6	2	−3	−3.3	−2	0.8	−4.5
Height (cm)	96	142.5	98	71	NE	NE	100	130	106
SD	−1.4	0.4	−2.3	2	NE	NE	−2.3	0.4	−1.7
Weight (kg)	15.5	43.5	13.2	9.325	NE	NE	15	27.5	14
SD	−0.3	2	−2.4	2	NE	NE	−1.5	0.4	−2.6
Developmental delay	Severe ID (no language)	Severe ID (no language)	Severe ID (no language)	Moderate ID	Moderate ID	Moderate ID	Severe ID (no language)	Moderate ID	Severe ID (no language)
Feeding problems	+ (gastrostomy tube)	Absent	+	Absent	Absent	Absent	Mild	Absent	Absent
Neurological signs									
Spastic tetraplegia	Asymmetrical	+	+	–			Asymmetrical		
Hemiplegia				+				+	
spastic diplegia					+	+			+
Major orofacial dyspraxia	Absent	absent	absent	+	+	+	absent	absent	+
Epilepsy									
Seizures type	infantile spasms	Focal epilepsy	Myoclonic and multifocal seizures	Focal epilepsy	Absent	Absent	Focal (Right temporal) epilepsy	Focal (Left temporal)	Absent
Refractory	Refractory	controlled	Refractory	Controlled			Refractory	Controlled	
Age of seizure onset (months)	5	NE	5	60	N/A	N/A	3	4	N/A
Ophthalmological signs									
Normal Ocular fundus	Normal fundus	Normal fundus	Normal fundus	Normal fundus	Normal fundus	Normal fundus	Normal fundus	Normal fundus	Normal fundus
Small/tilted optic discs	+	–	–	–	–	–	–	–	–
Myopia	–	–	–	–	–	–	–	–	–
Amblyopia/ Hemianopsia	intermittent eye fixation	–	–	–	–	–	–	–	–
Other		Esotropia	Bilateral cataract						

(*continued on next page*)

Table 1 (*continued*)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
CK levels	ND	Normal	Mildly elevated 230 UI/l	Normal	Normal	Normal	Normal	Normal	Normal
Renal ultrasound	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Hemolytic anemia	–	–	–	–	–	–	–	–	–

Abbreviations: ID = intellectual disability; m = months; SD standard deviation; NE = not evaluated; N/A not applicable; Head circumference (microcephaly was defined as < 2 SD score according to country-specific control cohorts; WHO child growth standards and the Fenton growth chart for preterm infants); *: variant of unknown significance.

samples from the following online databases: dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), ESP (http://evs.gs.washington.edu/EVS/) and gnomAD http://gnomad.broadinstitute.org/ (123,136 exomes and 15,496 genomes from unrelated individuals). All candidate variants were analyzed by the Alamut® Visual software using four pathogenicity prediction tools (PolyPhen-2, SIFT, MutationTaster and align GVGD) and *in silico* tools of evaluating their putative effect on RNA splicing (MaxEntScan, NNsplice, and HumanSplicingFinder).

3. Results

3.1. COL4A1/COL4A2 molecular screening data

One *de novo* missense mutation of *COL4A1* was identified in patient 3, affecting a highly conserved Gly residue in the Gly-X-Y repeat of the triple helical domain (c.3715 G > A, p.(Gly1239Arg)). This mutation was previously reported in a family in which a child had prenatal intracranial haemorrhage, porencephaly and hemolytic anemia, and her father had HANAC (Takenouchi et al., 2015). Two *COL4A2* mutations were found, respectively in one familial case (Patient 1) and one sporadic patient (Patient 2). The first one was a missense mutation (c.4129G > A, p.(Gly1377Arg)) also affecting a Gly residue in a Gly-X-Y repeat of the collagen triple helical domain of *COL4A2*. This mutation was inherited from the mother whose cerebral MRI showed periventricular white matter hypersignals, without any neurological symptom. The maternal grandfather of this proband died at 40 years of age from cerebral haemorrhage. In addition, a previous pregnancy of the mother's proband was interrupted due to fetal cerebral haemorrhage; DNA analysis of this fetus showed the c.4129G > A mutation. The second mutation was a splice site mutation (c.1776 + 1G > A) that disrupts a consensus donor splice site and is predicted to lead to exon 24 skipping according to *in silico* prediction tools (cDNA was not analyzed). This variant was inherited from the proband's asymptomatic mother who did not undergo cerebral MRI. The predicted consequence of exon 24 skipping is the creation of a stop codon (p.(Glu558*)). All 3 mutations are typical of *COL4A1/COL4A2* pathogenic mutations and are predicted to be pathogenic based on ACMG criteria (Table 1) (Richards et al., 2015).

In three cases, *COL4A1* variants were identified but their pathogenicity could not be definitely established and they were classified as variants of unknown significance. Variants of patient 4 (c.4727 C > T/p.Ser1576Leu) and patient 7 (IVS19 -37T > A) were inherited from an asymptomatic father. These variants affect neither a Gly triple helix residue nor a consensus splice site. These variants have a low prevalence/absence in controls (less than 1/10000 alleles) but nonetheless were considered as variants of unknown significance. Patient's 5 variant (p.(Pro530Ser)) was inherited from an asymptomatic mother and has a prevalence higher than 1/1000 in control databases, strongly suggesting that it is not a pathogenic variant. We did not identify any variant in the remaining 3 patients. In addition, no copy number alteration of COL4A1/COL4A2 coding exons was detected in the 3 patients who underwent QMPSF screening (patients 5, 7 and 8).

3.2. Clinical information

Clinical details for our cohort are summarized in Table 1, with representative brain images shown in Figs. 1 and 2. All patients but one were born following a full-term pregnancy and normal delivery. No patients reported a history of maternal hypertension or preeclampsia, alloimmune thrombocytopenia or bleeding during pregnancy.

All patients had a normal birth weight. OFC circumference was normal in all cases except one patient (patient 1) who had congenital microcephaly with an OFC > −3 standard deviations (SD) below the mean. At the most recent evaluation, postnatal microcephaly was noted in all patients from −2.5 to −3 SD, except in one. All patients presented with significant delay in motor skills and mild to severe

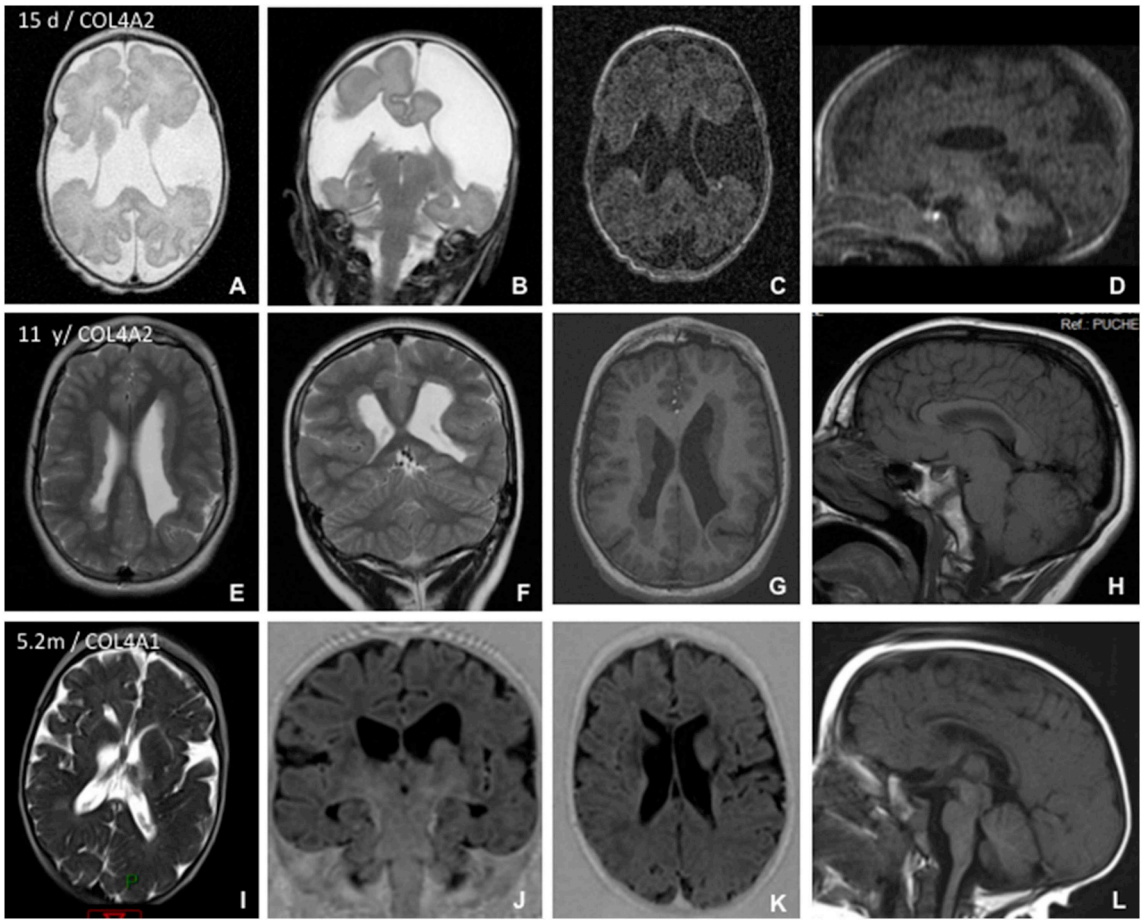

Fig. 1. Representative MRI section showing brain malformations in patients with *COL4A1* and *COL4A2* mutations.
Bilateral open lip asymmetrical schizencephaly with ventricular dilatation (A,B). Cortex overlying the cleft is polymicrogyria (A, B). No stigma of haemorrhage (A, C) and the white matter is normal (B, C). Corpus callosum is normal (D) in patient 1 aged 15 days with *COL4A2* mutation.
Extensive nodular periventricular heterotopia (E,F,G,H) with posterior polymicrogyria with bilateral and asymmetrical schizencephaly close-lip cleft (E,F,G); corpus callosum is normal (H) in patient 2 aged 11 years with *COL4A2* mutation.
Bilateral and asymmetrical polymicrogyria with unilateral porencephaly (I,J,K) and normal corpus callosum (H) in patient 3 aged 5 months, with *COL4A1* mutation.
Left column - Axial T2 weighted MRI; Second column - coronal T2 weighted axial MRI (except J T1 coronal); Third column- T1 weighted axial MRI and right column are mid sagittal MRI.

intellectual disability. Motor impairment ranged from spastic quadriplegia with virtually no motor development (4/9) to spastic diplegia (3/9) or hemiplegia (2/9). Two patients showed orofacial apraxia (or pseudobulbar palsy) with excessive drooling and no babbling.

Epileptic seizures were reported in 6/9 patients, being either focal (4/6) or generalized with infantile spasms or myoclonic seizures (2/6). Age of seizure onset varied from 3 months to 5 years of age. In patients with focal epilepsy, seizure control was achieved with monotherapy in all cases except in one. In contrast, the two patients with infantile spasms or myoclonic seizures developed refractory daily generalized seizures.

Ophthalmological features included congenital cataracts in patient 3, and esotropia in patient 2, but patient 1 had no eye signs, all these 3 patients had *COL4A1/COL4A2* mutations. Of note, no patients had anterior chamber abnormalities of the Axenfeld–Rieger type. None of our patients had evidence of clinical myopathy or a history of muscle cramps but one patient with a *COL4A1* mutation showed a mildly elevated creatine kinase. Similarly, we did not identify any patients with hemolytic anaemia, history of haematuria, renal failure or cystic lesions on renal ultrasound.

3.3. Brain MRI features

Bilateral asymmetrical schizencephaly was the most common MRI abnormality with a closed-lip defect in 5/6 (Table 2). The schizencephaly affected one or two cortical lobes (5/6), and involved most frequently the parietal and frontal lobes including the adjacent central fissure. Polymicrogyria lining the schizencephaly was observed in all cases, but was also found independently in the contralateral hemisphere in 5/6. Schizencephaly was associated with either ventriculomegaly or porencephaly in all cases (Figs. 1 and 2).

In one case (patient 2), schizencephaly was associated with a combination of polymicrogyria and nodular periventricular heterotopia (Fig. 1). Only one patient had an extracortical abnormality consisting of an absent septum pellucidum.

Two patients had white matter changes surrounding the polymicrogyric cortex/schizencephalic cleft suggestive of gliosis, but none had periventricular anomalies reminiscent of leukoencephalopathy. We did not observe evidence of old or recent haemorrhage in any of our patients. No posterior fossa abnormalities were noted.

4. Discussion

Central nervous system hallmarks of *COLA1A* and *COL4A2*

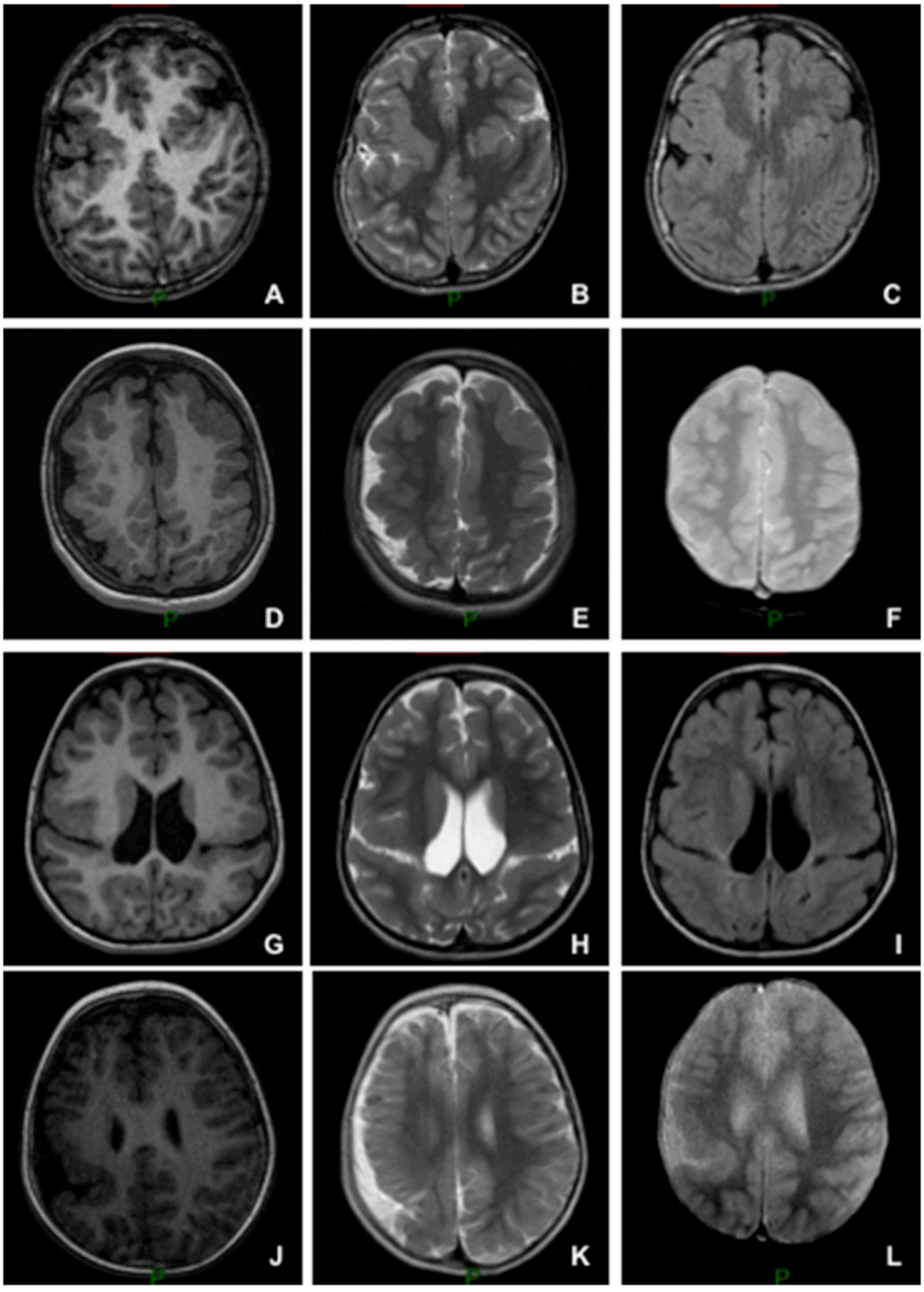

Fig. 2. Representative MRI section showing brain malformations in patients negative for *COL4A1* and *COL4A2* mutations or in patients with variants of unknown significance. Left column- Axial T1 weighted MRI; Middle column-T2 weighted axial MRI and Right column-FLAIR (C,I) and T2*-weighted gradient-echo image (WGRE) sequences (F,L).

A, B, C show typical bilateral perisylvian polymicrogyria with schizencephaly in Patient 5 (*COL4A1* variant of unknown significance) aged 9 years 5 months.

D, E, F show frontal and parietal polymicrogyria in patient 9, aged 6 years 4 months (no *COL4A1/COL4A2* mutation).

G, H, I show bilateral perisylvian polymicrogyria with large schizencephalic cleft in patient 6 aged 4 years 2 months (no *COL4A1/COL4A2* mutation).

J, K, L show unilateral parieto-occipital polymicrogyria with schizencephalic cleft in patient 7 aged 2 years 11 months (*COL4A1* variant of unknown significance).

mutations include porencephaly, schizencephaly, cerebral haemorrhage, deep lacunar infarcts, leukoencephalopathy, microbleeds, and intracranial carotid aneurysms (Vahedi and Alamowitch, 2011). In addition, *COL4A1* and *COL4A2* mutations are known to cause eye, muscle and kidney anomalies (Vahedi and Alamowitch, 2011). Ocular dysgenesis can occur in the anterior (Rieger syndrome, cataracts, microcornea, microphthalmia) and posterior portions of the eye (retinal arterial tortuosity, retinal haemorrhages and optic nerve hypoplasia) (Coupry et al., 2010; Gould et al., 2006; Sibon et al., 2007; Vahedi et al., 2003). Recently, Yoneda and coll. further expanded the spectrum of *COL4A1* mutations and included schizencephaly, pontocerebellar atrophy, focal cortical dysplasia, and haemolytic anemia (Yoneda et al., 2013).

Herein, we identified two pathogenic *COL4A2* and one pathogenic *COL4A1* mutations in 3 unrelated patients with schizencephaly and associated cortical malformations ranging from polymicrogyria to subependymal and subcortical heterotopia without any sign of haemorrhage or calcification. In addition, we provide new evidence that *COL4A2* mutations also cause schizencephaly. Finally, our data allow us to identify some distinctive features of *COLA1A/COL4A2* mutation related schizencephaly that differ from previously reported cases, probably because of the extreme precocity of the brain insult during development. Three out of nine patients with complex cortical malformations in which schizencephaly and polymicrogyria were the main features, were found to carry *COL4A1/COL4A2* mutations. This prevalence is lower than the previously reported 50% (Yoneda et al., 2013). All patients with schizencephaly were either severely or moderately affected with spastic quadriplegia and absent language. The

Table 2
Summary of MRI findings.

Patient number/ Age at MRI	*COL4A1/COL4A2* mutation	Schizencephaly	Porencephaly/ Ventriculomegaly	Cortical malformation external to the Schizencephaly	Associated CNS anomalies		
					White matter changes	Septum Pellucidum/ Corpus Callosum	Hemosiderin deposition (T2* sequence)
1/15 d	*COL4A2* mutation	Bilateral asymmetric Open lip (P + F)	Minor unilateral	Bilateral asymmetrical PMG	(–)	(–)	(–)
2/11.3 y	*COL4A2* mutation	Bilateral Asymmetric close lip (T + O lt > R)	Bilateral asymmetrical	Bilateral Subcortical curvilinear and subependymal nodular heterotopia Left posterior parietal pachygyria, and left occipital and right parieto-occipital PMG	(–)	(–)	(–)
3/5.2 m	*COL4A1* mutation	Bilateral Asymmetric close lip Lt > Rt (F lt + P Rt)	Unilateral	(–)	(–)	(–)	(–)
4/8.5 m	Unknown significance	Bilateral close lip (P + F)	Bilateral Mildly asymmetrical	(–)	Mildly delayed	(–)	(–)
5/9.5 y	Unknown significance	Bilateral close lip (Rt P + F)	Ventricular dilatation	(–)	(–)	Dysmorphic CC	(–)
6/4 y 2 m	Negative	Bilateral close lip (Rt P + F)	Ventricular dilatation	Bilateral PMG	Minor gliosis	(–)	(–)
7/2y 11 m	Unknown significance	Unilateral close lip (Lt T)	Unilateral	Unilateral PMG	(–)	(–)	(–)
8/11 m 1 d	Negative	(–)	(–)	Bilateral Asymmetrical PMG (Rt > Lt)	(–)	(–)	(–)
9/5y 11 m	Negative	Unilateral close lip (P Lt)	Minor unilateral	Parietal PMG contralateral to SCZ	(–)	CC Thin and short	(–)

Abbreviations: y = years; m = months; d = day. Rt = Right, Lt = Left; F = frontal, P = parietal, O = occipital, T = temporal; CNS = central nervous system; PMG = Polymicrogyria, CC = Corpus callosum; SP = septum pellucidum; SCZ = schizencephaly (–) = absent.

severe neurodevelopmental outcome described here is similar to Yoneda's series but is worse than the outcome reported in large cohorts of schizencephaly patients (Barkovich and Kjos, 1992; Packard et al., 1997). This difference is not related to the inclusion of patients with large cortical defects usually suspected to contribute to neurodevelopment dysfunction, since most patients reported had close lip schizencephaly either bilateral or unilateral affecting one or two lobes. Alternatively, this may reflect the broad range of cellular processes associated with *COL4A1 or COL4A2* mutations, affecting integrity, stability and functionality of basement vascular membranes during brain development (Gould et al., 2005; Poschl et al., 2004). On the other hand, one patient with mixed heterotopia in addition to bilateral polymicrogyria was found to carry a *COL4A2* mutation, which to our knowledge, is the first such reported case. Remarkably, the complex cortical malformation was associated with bilateral asymmetrical schizencephaly that is rarely observed in cases of periventricular heterotopias (Wieck et al., 2005). Extra-neurological features such as haemolytic anemia, elevated CK levels previously reported as hallmarks of *COL4A1* related disorders were not observed in our series, reinforcing that such clinical and biological features are suggestive but not necessary for the diagnosis (Yoneda et al., 2013).

COL4A1A-related disorders are characteristically, but not exclusively, associated with white matter changes consisting in periventricular leukomalacia and subtle periventricular, basal ganglia and/or deep white matter calcifications (Livingston et al., 2011, 2013). Intracranial calcifications in *COL4A1* mutations are undoubtedly dystrophic since they occur in areas where foetal haemorrhages lead to cell death then mineralization, without any vascular calcification (McCartney and Squier, 2014). Of interest, none of the *COL4A1* and *COL4A2* related cortical malformation cases reported here showed calcification, white matter changes, nor microbleeds on T2* weighted sequences. The absence of haemorrhagic features clearly contrasts with the previously reported cases with *COL4A1* related schizencephaly (Garel et al., 2013; Lichtenbelt et al., 2012; Meuwissen et al., 2015; Vermeulen et al., 2011; Yoneda et al., 2013). The early onset of the insult before 24 weeks of gestation probably accounts for the specific presentation of brain malformation, combining schizencephaly with or without porencephaly, and may also explain the possible disappearance of signs of intraparenchymal haemorrhage. Similarly, the early onset of cerebral insults, together with the extent of the lesions, probably accounts for the progressive microcephaly seen in all our cases.

5. Conclusion

An association between *COL4A1* mutations and schizencephaly, particularly with microbleeds or intracranial calcifications was first identified by Yoneda et al., in 2013 (Yoneda et al., 2013). The present study of *COL4A1/2* mutations associated brain malformations ranging from bilateral open-lip schizencephaly to subependymal heterotopia supports the same pathophysiological mechanisms for the two conditions. In addition, we stress that ophthalmological malformations in mutated patients are frequent, although not diagnostic, and microbleeds, intracranial calcification as well as haemolytic anemia and elevated CK levels are uncommon. Our results further demonstrate the importance of genetic testing not only of *COL4A1* but also *COL4A2* mutations in children with cortical malformations that include at least uni- or bilateral schizencephaly with overlying polymicrogyria.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2018.10.004.

References

Barkovich, A.J., 2000. Morphologic characteristics of subcortical heterotopia: MR imaging study. AJNR Am. J. Neuroradiol. 21 (2), 290–295.
Barkovich, A.J., Kjos, B.O., 1992. Schizencephaly: correlation of clinical findings with MR characteristics. AJNR Am. J. Neuroradiol. 13 (1), 85–94.
Bejar, R., Vigliocco, G., Gramajo, H., Solana, C., Benirschke, K., Berry, C., Coen, R., Resnik, R., 1990. Antenatal origin of neurologic damage in newborn infants. II. Multiple gestations. Am. J. Obstet. Gynecol. 162 (5), 1230–1236.
Coupry, I., Sibon, I., Mortemousque, B., Rouanet, F., Mine, M., Goizet, C., 2010. Ophthalmological features associated with COL4A1 mutations. Arch. Ophthalmol. 128 (4), 483–489.
Curry, C.J., Lammer, E.J., Nelson, V., Shaw, G.M., 2005. Schizencephaly: heterogeneous etiologies in a population of 4 million California births. Am. J. Med. Genet. 137 (2), 181–189.
Garel, C., Rosenblatt, J., Moutard, M.L., Heron, D., Gelot, A., Gonzales, M., Mine, E., Jouannic, J.M., 2013. Fetal intracerebral hemorrhage and COL4A1 mutation: promise and uncertainty. Ultrasound Obstet. Gynecol. Official J. Int. Soc. Ultrasound Obstet. Gynecol. 41 (2), 228–230.
Gould, D.B., Phalan, F.C., Breedveld, G.J., van Mil, S.E., Smith, R.S., Schimenti, J.C., Aguglia, U., van der Knaap, M.S., Heutink, P., John, S.W., 2005. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. Science 308 (5725), 1167–1171.
Gould, D.B., Phalan, F.C., van Mil, S.E., Sundberg, J.P., Vahedi, K., Massin, P., Bousser, M.G., Heutink, P., Miner, J.H., Tournier-Lasserve, E., John, S.W., 2006. Role of COL4A1 in small-vessel disease and hemorrhagic stroke. N. Engl. J. Med. 354 (14), 1489–1496.
Govaert, P., 2009. Prenatal stroke. Semin. Fetal Neonatal Med. 14 (5), 250–266.
Kuo, D.S., Labelle-Dumais, C., Gould, D.B., 2012. COL4A1 and COL4A2 mutations and disease: insights into pathogenic mechanisms and potential therapeutic targets. Hum. Mol. Genet. 21 (R1), R97–R110.
Larroche, J.C., Droulle, P., Delezoide, A.L., Narcy, F., Nessmann, C., 1990. Brain damage in monozygous twins. Biol. Neonate 57 (5), 261–278.
Larroche, J.C., Girard, N., Narcy, F., Fallet, C., 1994. Abnormal cortical plate (polymicrogyria), heterotopias and brain damage in monozygous twins. Biol. Neonate 65 (6), 343–352.
Leventer, R.J., Jansen, A., Pilz, D.T., Stoodley, N., Marini, C., Dubeau, F., Malone, J., Mitchell, L.A., Mandelstam, S., Scheffer, I.E., Berkovic, S.F., Andermann, F., Andermann, E., Guerrini, R., Dobyns, W.B., 2010. Clinical and imaging heterogeneity of polymicrogyria: a study of 328 patients. Brain J. Neurol. 133 (Pt 5), 1415–1427.
Lichtenbelt, K.D., Pistorius, L.R., De Tollenaer, S.M., Mancini, G.M., De Vries, L.S., 2012. Prenatal genetic confirmation of a COL4A1 mutation presenting with sonographic fetal intracranial hemorrhage. Ultrasound Obstet. Gynecol. Official J. Int. Soc. Ultrasound Obstet. Gynecol. 39 (6), 726–727.
Livingston, J., Doherty, D., Orcesi, S., Tonduti, D., Piechiecchio, A., La Piana, R., Tournier-Lasserve, E., Majumdar, A., Tomkins, S., Rice, G., Kneen, R., van der Knaap, M., Crow, Y., 2011. COL4A1 mutations associated with a characteristic pattern of intracranial calcification. Neuropediatrics 42 (6), 227–233.
Livingston, J.H., Stivaros, S., van der Knaap, M.S., Crow, Y.J., 2013. Recognizable phenotypes associated with intracranial calcification. Dev. Med. Child Neurol. 55 (1), 46–57.
McCartney, E., Squier, W., 2014. Patterns and pathways of calcification in the developing brain. Dev. Med. Child Neurol. 56 (10), 1009–1015.
Meuwissen, M.E., Halley, D.J., Smit, L.S., Lequin, M.H., Cobben, J.M., de Coo, R., van Harssel, J., Sallevelt, S., Woldringh, G., van der Knaap, M.S., de Vries, L.S., Mancini, G.M., 2015. The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. Genet. Med. 17 (11), 843–853.
Nabavizadeh, S.A., Zarnow, D., Bilaniuk, L.T., Schwartz, E.S., Zimmerman, R.A., Vossough, A., 2014. Correlation of prenatal and postnatal MRI findings in schizencephaly. AJNR Am. J. Neuroradiol. 35 (7), 1418–1424.
Packard, A.M., Miller, V.S., Delgado, M.R., 1997. Schizencephaly: correlations of clinical and radiologic features. Neurology 48 (5), 1427–1434.
Poschl, E., Schlotzer-Schrehardt, U., Brachvogel, B., Saito, K., Ninomiya, Y., Mayer, U., 2004. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development 131 (7), 1619–1628.
Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H.L., Committee, A.L.Q.A., 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet. Med. 17 (5), 405–424.
Sibon, I., Coupry, I., Menegon, P., Bouchet, J.P., Gorry, P., Burgelin, I., Calvas, P., Orignac, I., Dousset, V., Lacombe, D., Orgogozo, J.M., Arveiler, B., Goizet, C., 2007. COL4A1 mutation in Axenfeld-Rieger anomaly with leukoencephalopathy and stroke. Ann. Neurol. 62 (2), 177–184.
Takenouchi, T., Ohyagi, M., Torii, C., Kosaki, R., Takahashi, T., Kosaki, K., 2015. Porencephaly in a fetus and HANAC in her father: variable expression of COL4A1 mutation. Am. J. Med. Genet. 167A (1), 156–158.
Vahedi, K., Alamowitch, S., 2011. Clinical spectrum of type IV collagen (COL4A1) mutations: a novel genetic multisystem disease. Curr. Opin. Neurol. 24 (1), 63–68.
Vahedi, K., Massin, P., Guichard, J.P., Miocque, S., Polivka, M., Goutieres, F., Dress, D., Chapon, F., Ruchoux, M.M., Riant, F., Joutel, A., Gaudric, A., Bousser, M.G., Tournier-Lasserve, E., 2003. Hereditary infantile hemiparesis, retinal arteriolar tortuosity, and leukoencephalopathy. Neurology 60 (1), 57–63.
Vermeulen, R.J., Peeters-Scholte, C., Van Vugt, J.J., Barkhof, F., Rizzu, P., van der Schoor, S.R., van der Knaap, M.S., 2011. Fetal origin of brain damage in 2 infants with a COL4A1 mutation: fetal and neonatal MRI. Neuropediatrics 42 (1), 1–3.
Wieck, G., Leventer, R.J., Squier, W.M., Jansen, A., Andermann, E., Dubeau, F., Ramazzotti, A., Guerrini, R., Dobyns, W.B., 2005. Periventricular nodular heterotopia with overlying polymicrogyria. Brain J. Neurol. 128 (Pt 12), 2811–2821.
Yakovlev, P.I., Wadsworth, R.C., 1946. Schizencephalies; a study of the congenital clefts in the cerebral mantle; clefts with hydrocephalus and lips separated. J. Neuropathol. Exp. Neurol. 5 (3), 169–206.
Yoneda, Y., Haginoya, K., Kato, M., Osaka, H., Yokochi, K., Arai, H., Kakita, A., Yamamoto, T., Otsuki, Y., Shimizu, S., Wada, T., Koyama, N., Mino, Y., Kondo, N., Takahashi, S., Hirabayashi, S., Takanashi, J., Okumura, A., Kumagai, T., Hirai, S., Nabetani, M., Saitoh, S., Hattori, A., Yamasaki, M., Kumakura, A., Sugo, Y., Nishiyama, K., Miyatake, S., Tsurusaki, Y., Doi, H., Miyake, N., Matsumoto, N., Saitsu, H., 2013. Phenotypic spectrum of COL4A1 mutations: porencephaly to schizencephaly. Ann. Neurol. 73 (1), 48–57.