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.3715G > 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...
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 COL4A1/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 Transcriptor™ 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 AmpFISTR 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>
<thead>
<tr>
<th>Patient</th>
<th>COL4A1 mutation</th>
<th>COL4A2 mutation</th>
<th>Sex</th>
<th>Birth (gestational week)</th>
<th>Birth weight (g)</th>
<th>Birth weight (SD)</th>
<th>Birth Head circumference (cm)</th>
<th>Mean age at investigation (y)</th>
<th>Growth parameters</th>
<th>Neurological signs</th>
<th>Epilepsy</th>
<th>Ophthalmological signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Absent</td>
<td>c.3715G &gt; A, p. (Gly1239Arg)</td>
<td>Female</td>
<td>36</td>
<td>1740</td>
<td>−2.16</td>
<td>29</td>
<td>4</td>
<td>48.5</td>
<td>Absent</td>
<td>Asymmetrical</td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td>Absent</td>
<td>Exon 24, c.1776+1G &gt; A, p?</td>
<td>Male</td>
<td>39.28571429</td>
<td>4000</td>
<td>−0.71</td>
<td>−3.21</td>
<td>11</td>
<td>51</td>
<td>+ (gastrostomy tube)</td>
<td>+</td>
<td>Focal epilepsy</td>
</tr>
<tr>
<td>Patient 3</td>
<td>c.4129G &gt; A, p. (Gly1377Arg)</td>
<td>Pathogenic</td>
<td>Female</td>
<td>36</td>
<td>2910</td>
<td>1.34</td>
<td>NE</td>
<td>5</td>
<td>46</td>
<td>Absent</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Patient 4</td>
<td>Pathogenic</td>
<td>c.4279C &gt; T, p. (Ser1576Leu)*</td>
<td>Male</td>
<td>39</td>
<td>4040</td>
<td>−0.71</td>
<td>−0.11</td>
<td>2</td>
<td>46</td>
<td>Absent</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Patient 5</td>
<td>Uncertain significance</td>
<td>c.1588C &gt; T, p. (Pro530Ser)*</td>
<td>Male</td>
<td>36</td>
<td>2920</td>
<td>1.42</td>
<td>−0.44</td>
<td>−0.44</td>
<td>48</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Patient 6</td>
<td>Absent</td>
<td>Exon 24, c.1776+1G &gt; A, p?</td>
<td>Female</td>
<td>38</td>
<td>3050</td>
<td>0</td>
<td>32</td>
<td>5</td>
<td>46</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Patient 7</td>
<td>c.1085-37T &gt; A*</td>
<td>Pathogenic</td>
<td>Female</td>
<td>39</td>
<td>3220</td>
<td>−0.04</td>
<td>31</td>
<td>5</td>
<td>48</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Patient 8</td>
<td>Absent</td>
<td>Exon 24, c.1776+1G &gt; A, p?</td>
<td>Male</td>
<td>39</td>
<td>3100</td>
<td>−0.58</td>
<td>34</td>
<td>5</td>
<td>54</td>
<td>Absent</td>
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<td></td>
</tr>
<tr>
<td>Patient 9</td>
<td>Absent</td>
<td>Exon 24, c.1776+1G &gt; A, p?</td>
<td>Male</td>
<td>36</td>
<td>2375</td>
<td>−0.81</td>
<td>31</td>
<td>5</td>
<td>46</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
</tbody>
</table>

(continued on next page)
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.3715G > 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.4727C > 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 both 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
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 COL4A1 and COL4A2
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 anaemia (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 COL4A1/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.

<table>
<thead>
<tr>
<th>Patient number/</th>
<th>Age at MRI (y/m/d)</th>
<th>C4COL4A1/2 mutation</th>
<th>Cortical malformation external to the Schizencephaly</th>
<th>White matter changes</th>
<th>Associated CNS anomalies</th>
<th>Septum Pellucidum/Corpus Callosum</th>
<th>Hemorrhages deposition (T2&quot; sequence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>6/2m</td>
<td>COL4A2 mutation</td>
<td>Bilateral symmetric closure lip</td>
<td>Bilateral asymmetrical Polymicrogyria</td>
<td>Bilateral symmetric Polymicrogyria</td>
<td>Bilateral symmetric Polymicrogyria</td>
<td>(−) (−) (−)</td>
</tr>
<tr>
<td>2/1</td>
<td>3.3y</td>
<td>COL4A1 mutation</td>
<td>Unilateral</td>
<td>Unilateral</td>
<td>Unilateral</td>
<td>Unilateral</td>
<td>(−)</td>
</tr>
<tr>
<td>3/5/2m</td>
<td>COL4A1 mutation</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>(−)</td>
</tr>
<tr>
<td>4/4.5m</td>
<td>COL4A2 mutation</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>(−)</td>
</tr>
<tr>
<td>5/5.5y</td>
<td>COL4A1 mutation</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>(−)</td>
</tr>
<tr>
<td>6/4.7y</td>
<td>COL4A1 mutation</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>(−)</td>
</tr>
<tr>
<td>7/8.5y</td>
<td>COL4A1 mutation</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>(−)</td>
</tr>
<tr>
<td>8/9.7y</td>
<td>COL4A1 mutation</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>(−)</td>
</tr>
</tbody>
</table>

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=septom

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 neurodevelopmental 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 anaemia, 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 leucomalacia 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 anaemia 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/ejmg.2018.10.004.

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