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CLINICAL REPORT

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Intracerebral hemorrhage in a neonate with an intragenic *COL4A2* duplication

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Abstract

Intracerebral hemorrhage is rare in term born neonates. Besides several non-genetic risk factors, pathogenic variants in *COL4A1* and *COL4A2* have been described to play a role in the pathophysiology of neonatal intracerebral hemorrhage. To the best of our knowledge, no intragenic *COL4A2* duplications have been reported in humans to date. We report a neonate with intracerebral hemorrhage and a de novo intragenic *COL4A2* duplication. Although it is not clear yet whether this genetic factor fully explains the clinical phenotype, it may have contributed at least as a risk factor for cerebral hemorrhage. Screening for intragenic *COL4A1* and *COL4A2* duplications as part of collagen IV diagnostics should be considered as part of the fetal and neonatal work-up for unexplained cerebral hemorrhages and to collect more evidence of the pathogenicity of this genetic mechanism.

KEYWORDS

antenatal intracerebral hemorrhage, *COL4A2*, neonatal intracerbral haemorrhage

1 | INTRODUCTION

Mutations in type IV collagen are an important cause of intracerebral hemorrhage, white matter abnormalities and porencephaly (Gould, Phalan et al. 2005). In 13% of the patient samples sent for *COL4A1* and *COL4A2* analysis, mostly because of porencephaly or infantile cerebral hemorrhage, a mutation could be detected (Meuwissen, Halley et al. 2015). Other associated neurologic findings include malformations of cortical development, for example, polymicrogyria and schizencephaly (Zagaglia, Selch et al. 2018).

Pathogenic variants in *COL4A1/2* are usually missense variants leading to glycine-substitution, predicted to have a dominant negative effect on collagen formation. Splice-site and frameshift mutations have also been described, suggesting that haploinsufficiency of one of the two genes may be an additional pathogenic mechanism (Lemmens, Maugeri et al. 2013). However, *COL4A1* loss-of-function variants identified during whole exome sequencing studies are often not associated with a known *COL4A1/2*-related phenotype, rendering the pathogenic effect of haploinsufficiency less obvious. A high de novo rate of variants was reported in a cohort of hereditary porencephaly cases, about 40% of the variants appeared de novo (Meuwissen, Halley et al. 2015).

Here, we report a neonate with extensive cerebral microbleeds and a de novo intragenic *COL4A2* duplication.

2 | CLINICAL REPORT

The patient was a female neonate who was the second child of Dutch, healthy parents. The parents were unrelated.

After a spontaneous conception, the pregnancy was uneventful with a normal none-invasive prenatal testing (NIPT) and a normal structural ultrasound at 20 weeks of gestation (with head circumference at p50). At 35 + 6 weeks of gestation fetal ultrasound was performed because of intrauterine growth restriction, showing a head circumference <p2.3,a small frontal horn cyst next to the right lateral ventricle and white matter abnormalities in the right frontal lobe within the distribution of the medullary veins of the white matter. There were no signs of intraventricular hemorrhage or ventricular dilatation (Figure 1a,b). TORCH screen was performed, excluding fetal infection.

She was born spontaneously at 38 gestational weeks with Apgar scores of 9, 10, and 10 (at 1, 5, and 10 min). Her birth weight was 2,580 g (−1.1 SD), and the head circumference 30.5 cm (−2.2 SD). She was admitted to the neonatal intensive care unit because of neonatal hyperbilirubinemia which required intensive phototherapy (3 days). There were no dysmorphic features. On neurologic examination she showed axial hypotonia, hypertonia of the legs, bilateral ankle clonus and bilateral spontaneously upgoing toes.

The postnatal cranial ultrasound scan showed hemorrhage in the nucleus caudatus, two focal lesions with increased echogenicity in the right basal ganglia suspect for either hemorrhage or calcification, and diffuse and inhomogeneous echogenicity of the periventricular white matter with localized cyst formation in the left parietal-occipital lobe (Figure 1c–f).

The brain MRI, performed at day three showed diffuse hemorrhagic lesions throughout both hemispheres, predominantly in the periventricular white matter, but also in the basal ganglia, and microbleeds in the mesencephalon and cerebellum (Figure 1g–j).

The MRI scan was repeated three weeks later showing partial resorption of the white matter hemorrhages, with volume loss of the white matter and basal ganglia. Remarkably, there was an increase in the cerebellar microhemorrhages (Figure 1k–l).

On follow-up, at the age of 6.5 months, weight was below normal (−3 SDS) with normal length (−1.5 SDS), and there was microcephaly with a head circumference of 38.2 cm (−4 SDS). Neurologic examination showed severe axial hypotonia and hypertonia of the limbs. Motor development was delayed with decreased variability in movement and slight asymmetry with an AIMS (Alberta Infant Motor Scale) test of $p < 5$. At the age of 9 months she developed myoclonic seizures. EEG showed bilateral synchronic peaks, followed by slow waves during 1–2 s, and interictal epileptiform discharges in the left

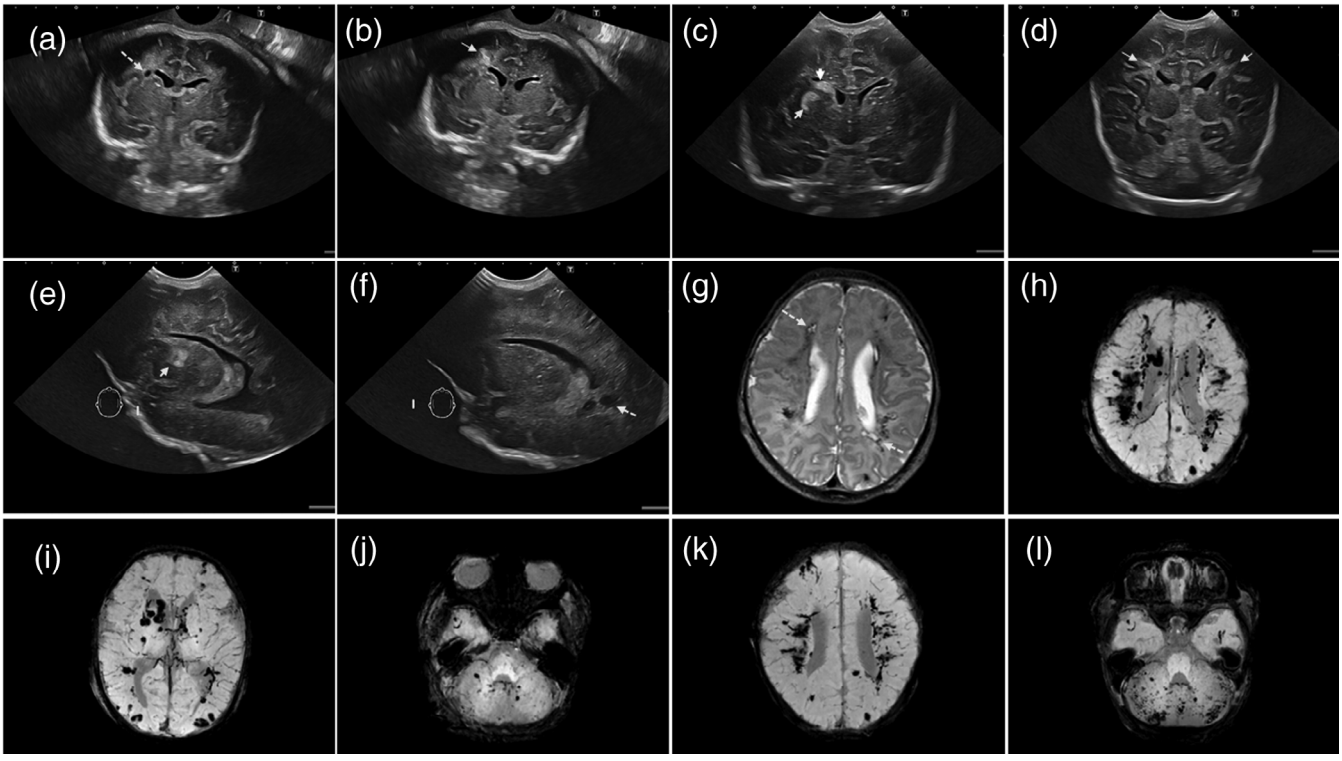

FIGURE 1 (a,b) Fetal neurosonography at 36 weeks of gestation showing white matter abnormalities in the right frontal lobe (coronal views), (c,d) coronal and (e,f) parasagittal postnatal ultrasound scan at day 2 showing hemorrhage in the nucleus caudatus, echogenicity in the basal ganglia and white matter, and white mater cysts (dotted arrow). MRI at 3 days of age, (g) T2-weighted image showing combination of hemorrhagic and cystic white matter lesions and minimum intensity projection showing extensive microbleeds periventricular (h), in basal ganglia (i), and in the cerebellum (j). Follow-up MRI around 4 weeks of age, minimum showing a slight reduction of microbleeds in the periventricular area (k) but an increase in the cerebellum (l). Arrows indicate abnormal echogenicity in white matter; dotted arrows white matter cysts, short arrow focal abnormality in basal ganglia, arrowhead intraventricular hemorrhage(for high resolution pictures, refer to the supplementary file)

occipital region. Levetiracetam was started with no effect on seizure frequency, after which she was switched to ethosuximide with moderate effect.

At the age of 12 months her head circumference remained stable at −3.8 SD, and signs of cerebral palsy further developed, for which baclofen treatment was started. During infections petechial rash was seen (Figure 2a). She also developed signs of cutis marmorata and vascular malformations on the soles of her feet (Figure 2b).

Additional investigations were performed in the neonatal period to unravel the cause of the extensive microbleeds. The platelet number, prothrombin time, and partial thromboplastin time were normal. The serum creatine kinase was repeatedly elevated (±1,257 U/L, as had been reported in *COL4A1* mutations before). Metabolic investigation (amino acids and organic acids in urine, and amino acids, carnitine profile, and sialotransferrins in serum and neonatal screening test) was normal. CMV screening in urine was negative. Feces was negative for rota-/adeno-/entero and parecho viruses. Ophthalmological investigation was normal. No venograms were performed.

3 | GENETIC ANALYSIS

Because of the imaging appearance of (antenatal development of) diffuse intracranial hemorrhages and cyst formation, a mutation in the *COL4A1/2* gene was suspected. Sanger sequencing of *COL4A1* (exon 1–52) and *COL4A2* (exon 2–48) in leucocyte DNA did not show pathogenic variants.

The Affymetrix CytoScanHD SNP-array showed an apparently de novo intragenic duplication of >99 kb (156 probes), containing exon 5–28 of the *COL4A2*-gene (coordinates of estimated minimally duplicated region: chr13: 111,030,406–111,129,816 bp [build GRCh37]). This duplication was not present in leucocyte DNA of the parents.

4 | DISCUSSION

Here, we describe a neonate with extensive cerebral microbleeds, predominantly in the white matter, but also in the deep grey matter, brain stem, cerebellum, cortex, and mesecenphalon. These lesions were partly visible on fetal ultrasound in the third trimester and confirmed on early postnatal cranial ultrasound and MRI. On follow-up she also developed subcutaneous vascular malformations, myoclonic epilepsy, and cerebral palsy. Genetic analysis showed an intragenic duplication in the *COL4A2* gene.

Full duplications and deletions of *COL4A1* and *COL4A2*, as part of larger deletions and duplications of 13q, mostly do not have porencephaly or intracerebral hemorrhage as a prominent feature (Wang et al. 2017), though a 44-year-old patient with cerebral small-vessel disease and a full duplication of *COL4A1* and *COL4A2* gene was reported (Renard, Mine et al. 2014). Intragenic duplications have been described in collagen IV (*COL4A5*) before (Renieri, Galli et al. 1995, Arrondel, Deschenes et al. 2004). We are not aware of any other pathogenic intragenic duplications in collagen genes.

We expect the duplication to have a dominant negative effect on the formation of collagen IV formation. Although it is not certain that this intragenic duplication is the sole cause of the cerebral microbleeds, the pre-test suspicion of a *COL4A1/2* mutation (based on the prenatal onset of intracranial hemorrhage and microcephaly; Zagaglia, Selch et al. 2018), the elevated CK which was previously reported in patients *COL4A1* variants (Tonduti, Pichiecchio et al. 2012) and the fact that the duplication was found to be de novo render a contribution of the duplication to the phenotype likely. Patients with pathogenic variants in *COL4A1/2* predominantly have microbleeds in basal ganglia, as was the case in our patient (Renard 2018). The presence of calcifications, as suggested by computed tomography, has also been described in patients with *COL4A1* mutations before (Livingston, Doherty et al. 2011). Importantly, more

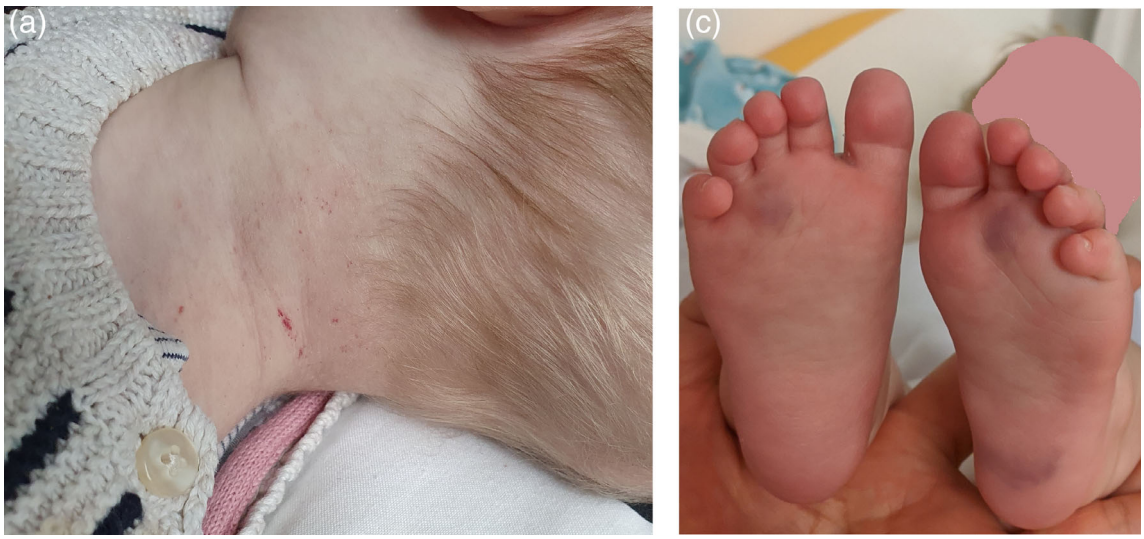

FIGURE 2 (a) Spontaneous petechial rash after viral infection. (b) vascular malformations on the foot soles [Color figure can be viewed at wileyonlinelibrary.com]

frequent causes of neonatal intracerebral hemorrhage including asphyxia and traumatic delivery are less likely to contribute to this case considering the uneventful delivery and already antenatal evidence of (hemorrhagic) white matter abnormalities. Coagulation, metabolic, and infectious testing did not show abnormalities, excluding other causes of intracranial hemorrhage and white matter injury.

There are arguments that do not support the pathogenicity of this variant as well. First, the exact location of the duplicated fragment is unknown and the functional consequences have not been established. Moreover, in our experience the extent of the microhemorrhages is more than usually seen in *COL4A1/2* sequence variants, suggesting that there may be another pathogenic mechanism than the dominant negative effect seen in sequence variants. Finally, rash and vascular malformations have not been reported in patients with *COL4A1/2* related disease before.

In conclusion, we report a neonate with diffuse cerebral microbleeds and a de novo intragenic duplication in *COL4A2*. Duplications in *COL4A2* may be a new, rare genetic mechanism hampering collagen IV function, potentially due to a dominant negative effect. Additional cases are needed to corroborate these findings.

CONFLICT OF INTEREST

Cacha M. P. C. D. Peeters-Scholte is founder of and consultant for Neurophyxia BV and she holds several patents and stocks of Neurophyxia BV. None of this work has a relation with the current manuscript. The other authors declare no potential conflict of interests.

AUTHOR CONTRIBUTIONS

S. Koene: writing of manuscript. C. M. P. C. D. Peeters-Scholte: correction of manuscript, preparation of figures. J. Knijnenburg: genetic analysis, correction of manuscript. L. S. de Vries: correction of manuscript. P. N. Adama van Scheltema: correction of manuscript, preparation of figures. M. E. Meuwissen: correction of manuscript. S. J. Steggerda: correction of manuscript, preparation of figures. G. W. E. Santen: correction of manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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