COL4A1 Mutation in Two Preterm Siblings with Antenatal Onset of Parenchymal Hemorrhage

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Objective: To report the presence of intracerebral hemorrhage and porencephaly, both present at birth, in two preterm infants with a mutation in the collagen 4 A1 gene.
Methods: Two preterm infants with antenatal intracerebral hemorrhage and established porencephaly, as well as their affected mother and grandfather, underwent neurological and ophthalmological examination and magnetic resonance imaging of the brain. Mutation analysis of the *COL4A1* gene was performed in the infants and in their mother.
Results: Both infants had a novel G1580R mutation in the *COL4A1* gene, encoding procollagen type IV α1. A history of mild antenatal trauma was present in the first but not in the second infant. Both preterm infants were asymptomatic at birth. The intracerebral hemorrhage and porencephaly were diagnosed with cranial ultrasound examination and were subsequently confirmed with magnetic resonance imaging. Leukoencephalopathy was present in the mother and in her father.
Interpretation: Mutation of the *COL4A1* gene appears to be a risk factor of antenatal intracerebral hemorrhage followed by porencephaly in the preterm newborn.

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Unilateral parenchymal hemorrhage is usually diagnosed in very-low-birth-weight preterm infants during the first week after birth. This type of hemorrhage usually follows a germinal matrix-intraventricular hemorrhage that can lead to impaired venous drainage of the medullary veins in the periventricular white matter and is either referred to as "venous hemorrhagic infarction" or "parenchymal hemorrhagic infarction."[1,2] Parenchymal hemorrhagic infarction will usually evolve into a porencephalic cyst over the next 4 to 6 weeks.[1,2] An *antenatal* PC after an antenatal parenchymal hemorrhage may occur in the context of neonatal alloimmune thrombocytopenia or a coagulopathy, for instance, von Willebrand's disease, factor V or X deficiency, and maternal warfarin use, but a definite cause is often not established.[3–6] Antenatal porencephaly has also been diagnosed in the context of thrombophilia, most often a heterozygosity for factor V Leiden mutation.[7,8] Antenatal porencephaly can also be preceded by antenatal trauma, even when this is mild or not directed at the uterine wall.[9,10]

Familial porencephaly with an autosomal dominant mode of inheritance was first reported almost half a century ago and usually presents in infancy or childhood with unilateral weakness.[11] In these cases, the presence of a porencephalic cyst is usually demonstrated by either computed tomography or magnetic resonance imaging (MRI). Although familial porencephaly is considered to be caused by an antenatal or perinatal parenchymal hemorrhage, this has never been documented with neonatal imaging, and in most cases, the neonatal period is reported as uneventful.[12–14] The affected parent may experience development of (asymptomatic) cerebral microbleeds, leukoencephalopathy, and symptomatic intracranial hemorrhage in later life.[12]

Autosomal dominant type 1 porencephaly has been linked to chromosome 13qter and was later found to

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be caused by a mutation in the collagen 4 A1 gene encoding procollagen type IV α1, a basement membrane protein expressed in all tissues.[15,16] Gould and colleagues[16] have shown that mice carrying mutations in the *COL4A1* gene have a high risk for intracerebral hemorrhage in the perinatal period, and that this complication can be avoided by surgical delivery. Based on these experimental findings, they suggested that prevention of birth trauma through surgical delivery may decrease the risk for intracerebral hemorrhage in humans with a *COL4A1* mutation.[17] We, however, report two preterm siblings with a documented antenatal parenchymal hemorrhage and an established porencephalic cyst at birth in the context of a mutation of the *COL4A1* gene.

Patients and Methods

Mutation Analysis of COL4A1

Written informed consent was obtained from both parents. Genomic DNA from the infants and from both parents was isolated from peripheral blood using standard protocols. The primers were designed to amplify the 52 exons including at least 50 bases of flanking genomic sequences based on the reference sequence of *COL4A1* as deposited in GeneBank (accession number for the messenger RNA NM_001845 and for the *COL4A1* gene Entrez GeneID 1282). Amplification reactions (exons 1–52) and sequence reactions were performed using DNA from Patients 1 and 2 according to Breedveld and coworkers.[18] Both parents and a panel of 150 ethnically matched control subjects (in total 300 alleles) were screened for the mutation with the use of specific primers amplifying *COL4A1* exon 50 (primers sequences are available on request). The mother's father died before permission for DNA analysis could be requested.

Case Reports

PATIENT 1. Patient 1, a male infant, was the first child of healthy nonrelated white parents. The pregnancy was complicated by urinary tract infections at 29 and 31 weeks gestation, treated with antibiotics on both occasions. At 23 weeks gestation, the mother had an elbow pushed into her abdomen while dancing, which resulted in abdominal pain and decreased fetal movements for a 24-hour period. No investigations were done at the time. At 29 weeks gestation, she developed uterine contractions, and was hospitalized for 1 week and treated with tocolysis. The cardiotocogram and fetal movements were considered normal. The contractions resolved, and the infant was subsequently born by a spontaneous vaginal delivery at 33 weeks and 5 days gestation. Apgar scores were 9 at 1 and 5 minutes. Birth weight was 1,990gm (50th centile), and head circumference was 32cm (50th centile). On day 1 he was transferred to a level three neonatal intensive care unit because he required ventilatory support and surfactant administration for respiratory distress. A routine cranial ultrasound examination on admission showed a large resolving hemorrhage in the left lateral ventricle with an ipsilateral porencephalic cyst and small cystic lesions in the periventricular white matter of the contralateral hemisphere (Figs 1A, B). A ventricular drain connected to a subcutaneous reservoir was inserted because of progressive ventricular dilatation, which eventually required a ventriculoperitoneal shunt. MRI was performed at 41 weeks postmenstrual age, confirming the porencephalic cyst and contralateral cystic lesions. Atrophy, probably related to Wallerian degeneration, was also observed in the left cerebral peduncle (see Figs 1C–E). A repeat MRI, performed at 24 months, showed high signal intensity suggestive of gliosis at the sites of previous periventricular cysts contralateral to the porencephalic cyst and persistence of the large porencephalic cyst. Renal ultrasound performed at 24 months did not show any cystic lesions. When he was last assessed at a corrected age of 18 months, he had a right-sided hemiplegia, a strabismus associated with a quadrant hemianopia, but no cataract or tortuosity of the retinal arteries. His developmental quotient was 68 on the Griffiths developmental assessment scale.[19]

PATIENT 2. During her second pregnancy, the mother experienced abdominal pain at 26 weeks gestation, was admitted to hospital for a week, and was subsequently discharged when premature contractions resolved. A female infant was born by spontaneous vaginal delivery at 31 weeks gestation. Apgar scores were 4 and 8 at 1 and 5 minutes, respectively. Birth weight was 1,320gm (25th centile), and head circumference was 29cm (50th centile). She did not experience respiratory problems but was referred to our neonatal intensive care unit because of her gestational age. Routine cranial ultrasound examination on admission showed a resolving hemorrhage in the left lateral ventricle with an ipsilateral porencephalic cyst and an area of echogenicity in the periventricular white matter of the contralateral hemisphere (Figs 2A, B). A ventricular drain connected to a subcutaneous reservoir was inserted because of rapidly progressive ventricular dilatation, which stabilized after 2 weeks. MRI was performed on day 7, confirming the head ultrasound findings. A small hemorrhage was seen in the left cerebellar hemisphere adjacent to the fourth ventricle. On diffusion-weighted imaging, increased signal intensity was present in the periventricular white matter bilaterally, suggestive of ongoing white matter injury (see Figs 2C–E). A repeat MRI at 43 weeks postmenstrual age showed absence of myelination of the posterior limb of the left internal capsule and atrophy of the left cerebral peduncle, probably caused by Wallerian degeneration. A few small cysts were noted in the previously hemorrhagic lesion in the right frontal white matter. Ophthalmological examination at the corrected age of 6 months showed a strabismus but no cataract or tortuosity of the retinal arteries. Renal ultrasound performed at 9 months of age did not show any cystic changes. She was noted to have an increased tone of the lower limbs, without any asymmetry and strabismus on a neurological examination.

Extensive laboratory investigations including a complete blood count, serum electrolytes, creatinine, liver enzymes, lipoprotein(a), and serum homocysteine were all within the reference range in both infants. An increased bleeding tendency was excluded by assessing the platelet count and activated partial thromboplastin time (APTT) and prothrombin time. Prothrombophilia screening including factor V Leiden

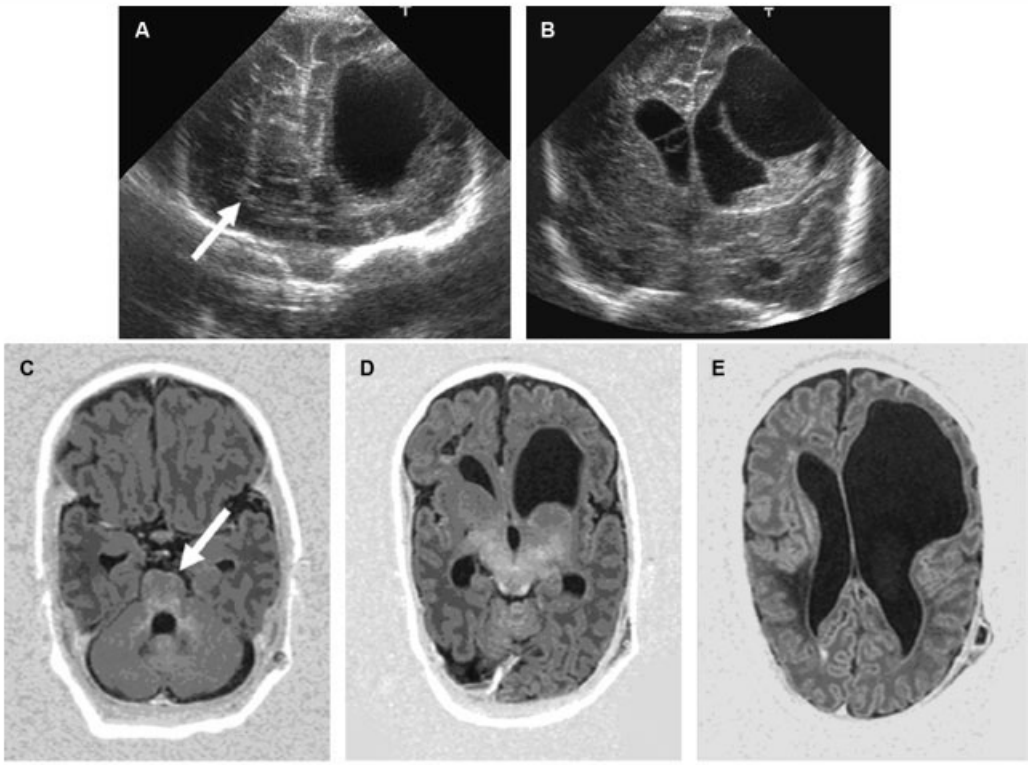

Fig 1. Day 5 cUS (A, B) and term-equivalent age magnetic resonance imaging (C–E) of Patient 1. The coronal ultrasound images show a small cyst in the right frontal periventricular white matter (arrow) *(A), and the large left-sided PC (B) with a midline shift to the right and a resolving clot in the left lateral ventricle. The axial inversion recovery sequence images show Wallerian degeneration* (arrow) *(C), several periventricular cysts in the right frontal periventricular white matter (D), and a porencephalic cyst (E).*

mutation, prothrombin gene mutation (G20210A) and MTHFR C677T mutation, lupus anticoagulant, anticardiolipin antibodies, and anti–B2GP1-IgG demonstrated no abnormalities. Platelet typing was performed in the parents, and neonatal alloimmune thrombocytopenia was excluded.

FAMILY HISTORY. The mother has an uneventful medical history, except for a tonsillectomy and a fracture of her left foot. She has not completed secondary education. General and neurological examination of the mother at the age of 23 years, 5 months after the birth of her second child, was unremarkable, except for a slight pupillary asymmetry. Ophthalmological examination did not show cataract or tortuosity of the retinal arteries. She did not have hematuria, and no cystic lesions were found with renal ultrasound. Her MRI showed mild ventricular dilatation, multiple hyperintense lesions in the periventricular white matter of both hemispheres (Figs 3A–C), but no dilated perivascular spaces or abnormalities on the gradient-echo images (T2*-weighted images) suggestive of (micro)bleeds.[20] Her father had a history of transient ischemic attacks at the age of 50 years and died at the age of 52 years after a severe intracerebral hemorrhage (see Fig 3F). He had no history of hypertension and had used carbasalate calcium and atorvastatin since the transient ischemic attacks. His MRI after the transient ischemic attacks showed porencephalic dilatation of the left lateral ventricle, as well as multiple hyperintense lesions in the periventricular white matter of both hemispheres (see Figs 3D, E). The MRI, performed in a regional hospital, did not include an echo-gradient sequence to detect microbleeds. The infant's paternal grandfather had a stroke during a mitral valve replacement when he was 66 years old.

Results

Genomic DNA sequencing of *COL4A1* from Patients 1 and 2 showed a heterozygous G>C change at nucleotide 4738 (c.G4738C) of exon 50, causing a glycine-to-arginine substitution at position 1580 of the procollagen-4 α1 chain (p.G1580R) (Fig 4). The same heterozygous missense mutation was present in DNA from the mother but not from the father (see Figs 3D–F). This change has not been described in other patients and was not present in 300 ethnically matched control chromosomes. The change was located in one of the Gly-X-Y repeats of *COL4A1* at the second C-terminal tandem repeated domain, which is highly conserved in type 4 procollagens, introducing the positively charged arginine instead (see Fig 4D). Mutations in this domain have been described in collagen 4–related human disease and are known to affect the stable conformation of the vascular basement membrane.[21] Therefore, we considered the G1580R substitution to be a pathogenic mutation. DNA of the ma-

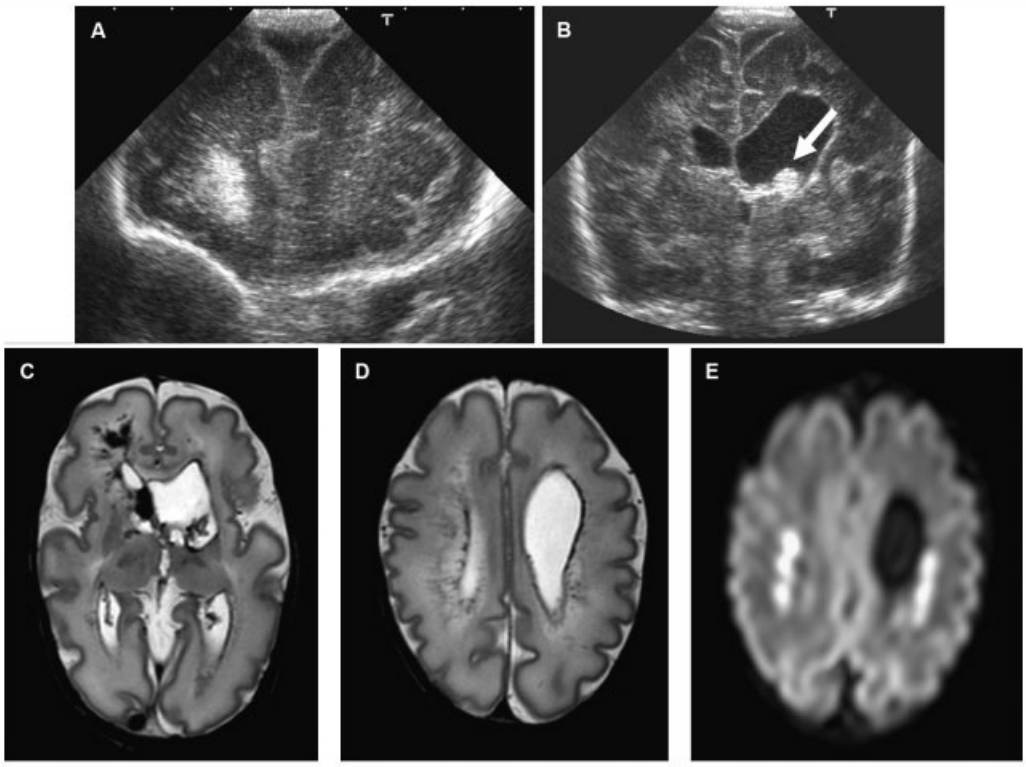

Fig 2. Day 1 cUS (A, B) and day 7 magnetic resonance imaging (C–E) of Patient 2. The coronal ultrasound images show an area of increased echogenicity in the right frontal periventricular white matter (A) and the large left-sided PC (B) with a small clot in the left lateral ventricle (arrow). *The axial T2 spin-echo sequence images show the presence of hemorrhage in the right frontal lobe and the right lateral ventricle (C), high and low signal intensity adjacent to the lateral ventricles and the left-sided PC (D), and high signal intensity on diffusion-weighted imaging in the periventricular white matter (E).*

ternal grandfather was not available for COL4A1 analysis.

Discussion

We report on a novel mutation in the *COL4A1* gene in a Dutch family presenting with autosomal dominant familial PC in two infants born preterm. This family shows that the mutation in the *COL4A1* gene carries a risk for development of intracerebral hemorrhage from fetal life into late adulthood, which can be associated with severe neurological sequelae and death. This is the first report of familial porencephaly diagnosed at birth, and in infants born prematurely, with the remains of the related hemorrhage still present in the lateral ventricle and in the first child in the porencephalic cyst. In both infants, lesions suggestive of hemorrhagic and/or ischemic injury to the periventricular white matter were also found in the contralateral hemisphere. Routine cranial ultrasound examinations in both infants and brain MRI in the second infant demonstrated resolving hemorrhages in the first days after birth, suggesting that the hemorrhage had occurred several weeks before their preterm birth. It is therefore unlikely that delivery by an elective caesarean section would have been able to prevent the parenchymal hemorrhage and subsequent evolution into a porencephalic cyst in these two infants, although this might have prevented additional injury in the second child, who had changes on diffusion-weighted imaging suggestive of recent injury. Possible antenatal trauma could be established only in the first infant, when his mother had incurred a mild abdominal injury at a gestational age of 23 weeks. Both pregnancies were complicated and resulted in preterm delivery with a diagnosis of a porencephalic cyst on routine head ultrasound examination. In case of a delivery at term, the diagnosis would probably have been made later in infancy, after presentation with unilateral weakness, at a stage when the hemorrhage would have had time to resolve.[22]

Gould and colleagues[16] first reported in mice and in humans the presence of a mutation in the procollagen type IV $\alpha 1$ that compromises the vascular basement membrane. They reported that cerebral hemorrhage occurred in all 20 mice pups after normal delivery, of which 14 were severe. By contrast, none of 26 surgically delivered mutant pups had a severe cerebral hemorrhage. Still, surgically delivered pups did occasionally have small hemorrhages visible through the skin, suggesting that even the pressure in utero was sufficient to cause these smaller lesions.[17] Based on these findings, it

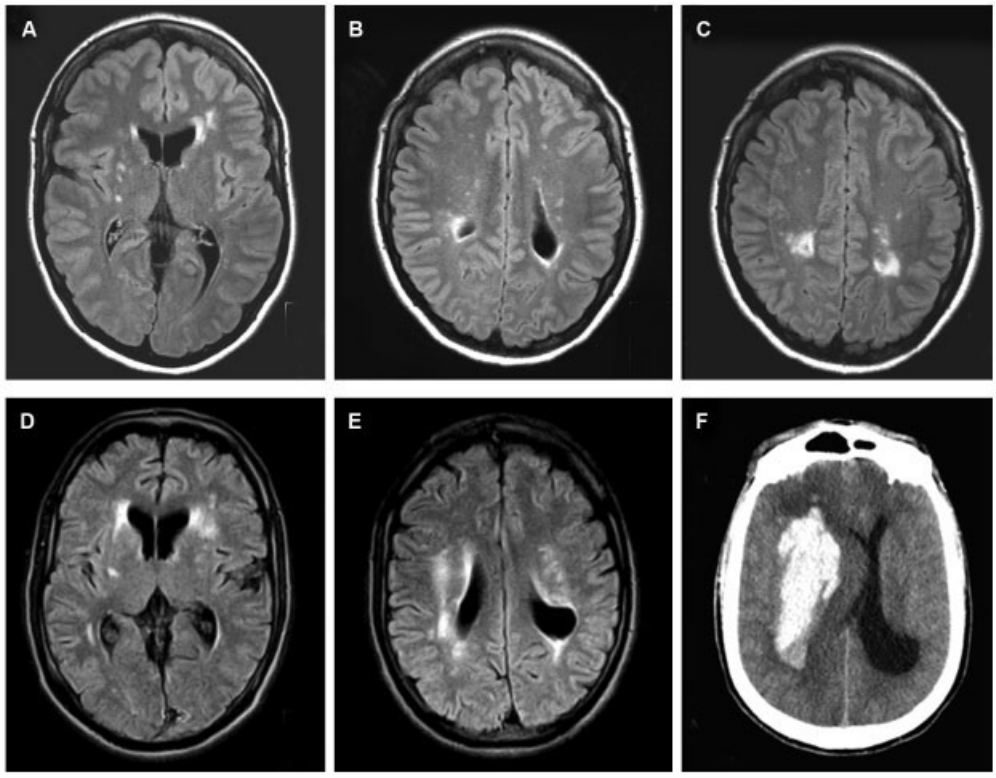

Fig 3. (A–C) Magnetic resonance imaging (MRI) of the mother. The axial fluid-attenuated inversion recovery (FLAIR) sequence images show mild porencephalic dilatation of the left lateral ventricle and bilateral gliotic changes in the periventricular white matter. (D–F) MRI and computed tomography of the maternal grandfather. The axial FLAIR sequence images (A, B) show mild ventricular dilatation, bilateral gliotic changes in the periventricular white matter, and porencephalic dilatation of the left lateral ventricle. A computed tomographic scan performed 2 years later (C) shows a large parenchymal hemorrhage.

was suggested that delivery by caesarean section to avoid birth trauma may prevent occurrence of a perinatal parenchymal hemorrhage in humans with *COL4A1* mutation.[17]

Of 19 patients with a *COL4A1* mutation that Vahedi and colleagues[13] reported, 13 presented with porencephaly and hemiplegia during infancy and only 3 had perinatal events. Two were postterm, and one had a prolonged second stage of delivery.[13] This also suggests that the lesions were acquired antenatally.

There are two additional findings in our family. Neither preterm delivery nor evidence of a hemorrhage at the time of diagnosis has been reported in association with this mutation. Furthermore, it was of interest that, in the second child, signal intensity changes were still present on diffusion-weighted imaging 2 weeks after delivery. The time course of diffusion changes in preterm infants is not well known and may persist for a longer period than in full-term infants, but it is also possible that the ongoing changes seen in the second sibling are associated with the *COL4A1* mutation and precede subsequent leukoencephalopathy. None of the previously reported cases was born prematurely. Because the *COL4A1* mutation we describe is new, it cannot be excluded that this new mutation carries an increased risk for preterm delivery and greater susceptibility to environmental trauma in the antenatal period than previously reported mutations in the same gene. Both pregnancies were complicated by repeated periods of preterm contractions, requiring hospital admission and tocolysis, and it is possible that these periods were experienced as "environmental trauma" for the fragile intracranial vasculature of these preterm infants. In contrast with the complications that occur early in the third trimester in both infants, their mother did not have a relevant medical history at the age of 23. She did not have retinal arterial tortuosity, but her MRI showed a mild porencephalic dilatation, possibly after cerebral infarction or hemorrhage. In view of recently published data, it is possible that she may experience intracerebral hemorrhage or infarction at a later age, similar to her father.[12] Mutations in *COL4A1* exons 24 and 25 have also been associated with nephropathy, intracranial aneurysms, and muscle cramps (HANAC [hereditary angiopathy with nephropathy, aneurysms, and cramps] syndrome). Performing cerebral magnetic resonance angiography and

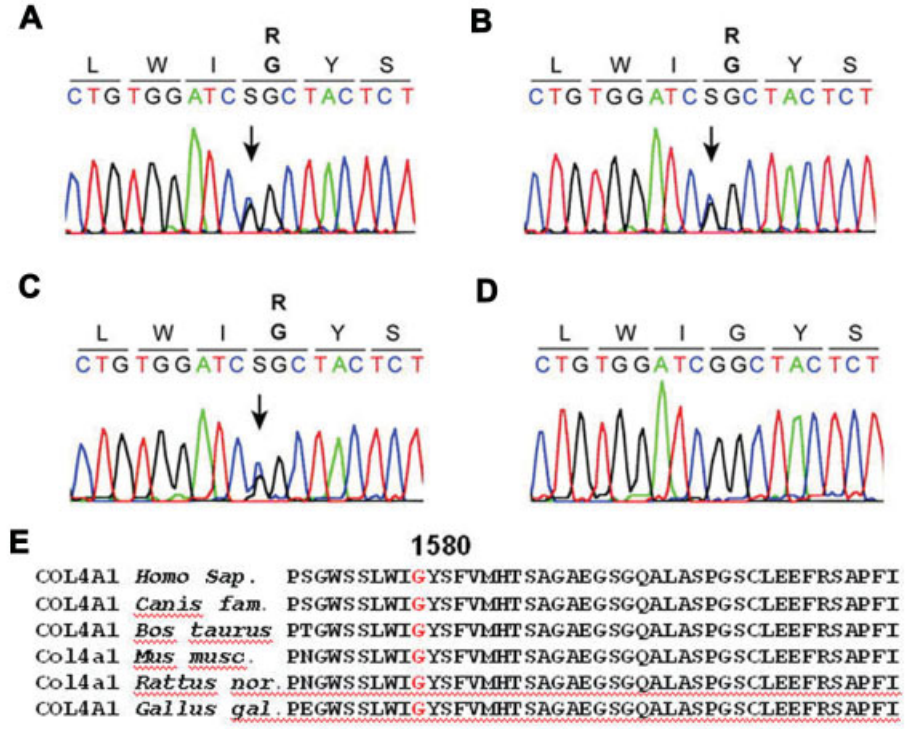

Fig 4. (A–E) Sequence electropherograms of the COL4A1 *mutation. Normal and mutated DNA and amino acid sequences are shown. (A, B) In Patients 1 and 2, the heterozygous G-to-C change at position 4738 (c.G4738C) in exon 50, resulting in a substitution of a glycine by arginine (p.G1580R), is indicated by the* arrow. *The same heterozygous mutation is found in the mother (C) but not in the father (D). (E) Amino acid sequence alignment of COL4A1 shows Glycin 1580 (red) in the C-terminal tandem repeated domain, highly conserved in type 4 procollagens.*

renal function tests is, therefore, indicated as well.[23] No nephropathy or evidence of aneurysms was found in our family, making the diagnosis of HANAC syndrome unlikely.

Mutation of the *COL4A1* gene is usually not suspected when a unilateral parenchymal hemorrhage or a porencephalic cyst are diagnosed during the neonatal period in a very-low-birth-weight infant. In most cases, the hemorrhage is not present at birth but tends to develop during the first few days of a complicated neonatal course. We suggest that testing for mutation of the *COL4A1* gene should be considered in preterm infants who develop a parenchymal hemorrhage, which is atypical in presentation, and in all infants who present with a porencephalic cyst at birth because our findings show that mutation of the *COL4A1* gene can be associated with antenatal parenchymal hemorrhage and neonatal porencephaly.

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