Atypical timing and presentation of periventricular haemorrhagic infarction in preterm infants: the role of thrombophilia

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ABBREVIATIONS

CSVT	Cerebrosinovenous thrombosis
cUS	Cranial ultrasonography
FVL	Factor V Leiden
GMH-IVH	Germinal matrix haemorrhage–intraventricular haemorrhage
MACS	Manual Ability Classification System
MRI	Magnetic Resonance Imaging
MTHFR	Methylenetetrahydrofolate reductase
PLIC	Posterior limb of the internal capsule
PVHI	Periventricular haemorrhagic infarction
TEA	Term-equivalent age

AIM Periventricular haemorrhagic infarction (PVHI) is a complication of preterm birth associated with cardiorespiratory instability. To date, the role of thrombophilia as a possible additional risk factor in infants with atypical timing and presentation of PVHI has not been investigated.
METHOD This was a retrospective cohort study of preterm infants who developed PVHI with an atypical timing and presentation either of antenatal onset or late in the postnatal course in the absence of a preceding sudden deterioration of their clinical condition. In infants with atypical PVHI mutation analysis of the factor V Leiden (G1691A), prothrombin (G20210A) gene, and C677T and A1298C polymorphisms in the *MTHFR* gene was performed, and plasma lipoprotein(a) and homocysteine levels were measured.
RESULTS Sixty-two preterm infants who presented with a PVHI were studied. Seventeen had an atypical presentation (seven males, 10 females; median birthweight 1170g [range 580–1990g]; median gestational age 30.6wks [range 28.7–33.7wks]). The typical PVHI group comprised 28 males and 17 females (median birthweight 1200g [range 670–2210g]; median gestational age 29.6wks [range 25.3–33.6wks]). Among the 17 infants with atypical presentation, the factor V Leiden mutation was found in seven infants (41%) as well as in the mothers of six of these seven infants; in one infant this was concomitant with a prothrombin gene mutation. A polymorphism in the *MTHFR* gene was also present in these infants. In two infants with an atypical presentation who were tested for this, a mutation in the *COL4A1* gene was found (reported previously). All but two of the infants with an atypical presentation developed spastic unilateral cerebral palsy.
INTERPRETATION An atypical presentation of PVHI in preterm infants tends to occur more often in the presence of thrombophilia. Testing of thrombophilia, especially factor V Leiden and prothrombin gene mutation, is recommended in these infants.

Periventricular haemorrhagic infarction (PVHI) is a complication of a germinal matrix haemorrhage–intraventricular haemorrhage (GMH-IVH) and is often associated with an adverse neurodevelopmental outcome.[1] While the incidence of cystic periventricular leukomalacia is decreasing, this is not the case with PVHI.[2,3] The incidence is 3 to 11% in infants born at less than 32 weeks gestational age and 5 to 8% for those with an extremely low birthweight.[4,5] PVHI is considered to be due to impaired drainage of the medullary veins in the periventricular white matter and tends to be preceded by an ipsilateral IVH or GMH. The PVHI may be associated with ischaemia of the white matter.[5]

PVHI usually develops during the first 24 to 72 hours after delivery in the context of preterm birth, low birthweight and haemodynamic instability.[5,6] Although the role of cerebral haemodynamic instability in the pathogenesis of PVHI is well established,[7–9] little is known about the possible role of mutations or polymorphisms, especially in genes encoding coagulation factors or collagen genes. These include factor V Leiden (FVL) mutation (G1691A), prothrombin mutation (G20210A) and C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene, elevated lipoprotein(a), and antithrombin, protein C and protein S deficiency. Homozygosity as well as heterozygosity of FVL mutation and the prothrombin mutation are associated with a hypercoagulable state,[10,11] whereas the C677T polymorphism in the MTHFR enzyme is associated with higher plasma homocysteine levels and has therefore been discussed as a risk factor for vascular disease.[12,13] Antithrombin, protein C and protein S are inhibitors of haemostasis and even partial deficiencies contribute to thromboembolic disease. The prothrombotic effect of elevated levels of lipoprotein(a) is considered to be due to a possible competition with plasminogen in fibrinolysis.

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An association between FVL and prothrombin gene mutation and perinatal stroke has been reported, but there is still no agreement about the association between perinatal stroke and the *MTHFR* polymorphisms.[13]

An association has also been suggested for FVL in the development of GMH-IVH[14] and for both the FVL and the prothrombin mutation in PVHI.[15,16] However, in a recent study by Petäjä et al.,[16] the cohort was rather small and PVHI was only diagnosed in a small number of newborn infants. Furthermore, no distinction was made between typical and atypical PVHI.

Mutations in *COL4A1*, a gene encoding a major basement membrane protein, have recently been shown to be associated with antenatal parenchymal haemorrhage with a porencephalic cyst already present at birth.[17,18]

The aim of this study was to investigate the presence of genetic mutations or polymorphisms in preterm infants with atypical timing and presentation of PVHI, with either an antenatal or a late neonatal onset.

What this paper adds

- Atypical timing and presentation of PVHI is not uncommon in preterm infants and should alert the clinician for associated problems.
- Thrombophilia, especially FVL mutation, is more common than expected in preterm infants with an atypical timing and presentation of PVHI.
- In preterm infants with a PVHI with atypical presentation or time of onset, further investigations are recommended, especially testing of FVL mutation.

METHOD

Patients

In a retrospective analysis, we studied preterm infants (<34wks gestational age) who had been admitted to the neonatal intensive care unit of the Wilhelmina Children's Hospital, Utrecht, the Netherlands, between January 2005 and December 2010 and who were diagnosed to have a PVHI on routine cranial ultrasonography (cUS). A distinction was made between a typical presentation of PVHI, where the onset is in the first 96 hours after birth as a complication of a GMH-IVH, and an atypical timing or presentation of PVHI. Atypical PVHI was defined when one of the following criteria was met: (1) presumed antenatal onset as shown on cUS less than 6 hours after birth and none of the following conditions: Apgar score at 5 minutes of less than 5, umbilical artery pH<7.0, need for resuscitation; (2) onset of PVHI more than 96 hours after birth in the absence of the following clinical conditions: patent ductus arteriosus, sepsis, vasopressor and ventilatory support, pulmonary haemorrhage and pneumothorax. Infants were not eligible for the study when a PVHI more than 96 hours after birth was related to an acute deterioration such as sepsis or need for mechanical ventilation. No ethical permission was required by the medical ethical committee of our hospital for this retrospective, anonymous study.

Clinical characteristics

Hospital charts of all infants in the study were reviewed for clinical and demographic information, including birthweight, gestational age, pre-eclampsia, chorioamnionitis, Apgar score at 1 and 5 minutes, arterial umbilical cord blood pH, need for emergency Caesarean section, respiratory and circulatory failure, patency of the ductus arteriosus, (sub)clinical seizures, and adverse neurological outcome.

Thrombophilic examination

In the infants with atypical PVHI, analysis for heterozygous or homozygous mutations of FVL (G1691A) and the prothrombin gene (G20210A) and C677T and A1298C polymorphisms in the *MTHFR* gene was performed by amplification and polymerase chain reaction analysis. Before the FVL mutation analysis, activated protein C resistance was measured and levels above 0.90 were considered as FVL negative. Plasma lipoprotein(a) and homocysteine levels were measured using nephelometry (Siemens Dade Behring BN II; Siemens, Deerfield, IL, USA) and a chemiluminescence competitive immunoassay (Siemens IMMULITE) respectively. Mothers of infants with a FVL or prothrombin gene mutation were tested as well.

Imaging

Cranial ultrasonography was part of routine clinical care. A first cUS was always performed at admission, additional cUS was performed two to three times during the first week of life, then once a week until discharge and again at 40 weeks postmenstrual age. PVHI was diagnosed when a triangular lesion was present in the periventricular white matter (Fig. 1a,b).

Magnetic resonance imaging (MRI) was performed either as soon as possible after birth and/or at term-equivalent age (TEA) to assess the (a)symmetry of the myelination in the posterior limb of the internal capsule (PLIC) in order to predict neurological outcome (Fig. 1c).[19]

Neurological outcome

Infants with spastic unilateral cerebral palsy (CP) were diagnosed according to the definition described by Rosenbaum et al.[20] at a corrected age of at least 18 months during the neurodevelopmental follow-up programme. The Manual Ability Classification System (MACS) was used to classify how infants older than 24 months with spastic unilateral CP use their hands when handling objects in daily activities and to define the severity of functional disability (where 1 is independent use and 5 is total assistance needed).[21]

Statistical analysis

Statistical analysis was performed with SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA) to identify differences between infants with typical and atypical presentation of PVHI. The Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables were used. Statistical significance was defined as $p<0.05$.

RESULTS

Between January 2005 and December 2010, 1782 preterm infants (<34wks gestational age) were admitted. Sixty-two infants (3.5%) were diagnosed to have PVHI on routine cUS. Among the 62 preterm infants 17 (27%) comprising seven males, 10 females (median birthweight 1170g [range 580–

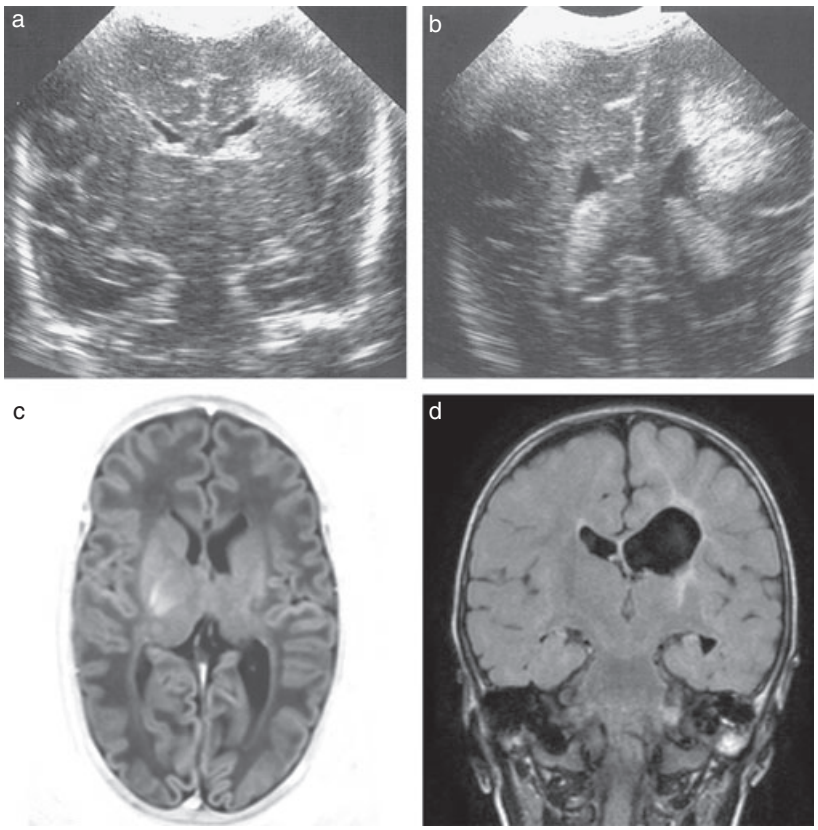

Figure 1: Female infant (case 4) born by emergency Caesarean section because of maternal haemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome; gestational age 30 weeks, birthweight 850g (p10), Apgar scores 6 and 9 at 1 minute and 5 minutes respectively; umbilical cord pH 7.27; mild infant respiratory distress syndrome; inotropic support on the first day. Cranial ultrasound (cUS) examination on admission: mild increase in echogenicity in the periventricular white matter, resolved by day 5. Postnatal course remained stable. (a, b) Repeat cUS examination on day 15 shows left-sided periventricular haemorrhagic infarction and an associated intraventricular haemorrhage grade I; (c) term-equivalent age magnetic resonance image (MRI) shows absence of myelination of the posterior limb of the internal capsule (PLIC) on the left side. (d) fluid-attenuated inversion recovery MRI at the age of 24 months shows a porencephalic cyst and periventricular gliosis adjacent to the cyst, affecting the PLIC on the left side. The infant developed spastic unilateral cerebral palsy on the right side. Coagulation profile: heterozygosity for factor V Leiden mutation and homozygosity for *MTHFR* A1298C polymorphism; the haemorrhage time, prothrombin time, and thrombin time were within normal limits. The plasma lipoprotein(a) level at 170mg/L was within the normal range.

1990g]; median gestational age 30.6wks [range 28.7–33.7wks]) had an atypical presentation (Fig. 2). Forty-five infants had typical PVHI presentation (28 males, 17 females; median birthweight 1200g [range 670–2210g]; median gestational age 29.6wks [range 25.3–33.6wks]).

Timing of cUS

The first cUS was performed within 6 hours after birth in the 45 infants with a PVHI who were inborn. Of these 45 infants, seven had an antenatal PVHI and two an established porencephalic cyst present on the first cUS. Thirty of these 45 inborn infants developed a PVHI within the first 96 hours, which was in the context of established risk factors, and six had a late-onset PVHI (>96h) without preceding deterioration of the clinical condition or haemodynamic instability.

Seventeen of the infants with a PVHI were outborn and did not have their first cUS within 6 hours after birth but between day 1 and 3 after birth. Fifteen of these infants could be classified as having 'typical' PVHI. Nine of these 15 infants developed PVHI before day 4 and the other six did show a PVHI on their first scan but in the context of established risk factors. In two of the 17 infants with 'atypical' PVHI, serial cUS was performed in a level II hospital, and a progression to PVHI after 96 hours was seen. The PVHI was the reason for the referral to our level III unit in these two infants.

The clinical characteristics of all infants with a PVHI are summarized in Table I.

In all but one of the infants with typical presentation, PVHI was secondary to GMH-IVH. One infant had grade I IVH, 40 had grade II IVH, and three had grade III IVH. In those with an atypical presentation, five infants had no GMH-IVH, one had grade I IVH, 10 had grade II IVH, and one had grade III IVH (Table I).

MRI

An early MRI (range 3–23d) was performed in 13 of the 17 infants with atypical PVHI and a TEA MRI in 15 of them; no MRI was available in one infant. Of the 45 infants with a

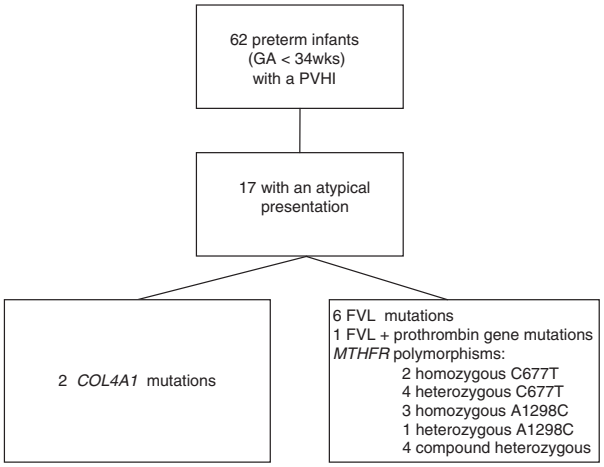

Figure 2: Flow diagram of 62 infants born preterm with periventricular haemorrhagic infarction (PVHI). FVL, factor V Leiden gene; GA, gestational age; *MTHFR*, methylenetetrahydrofolate reductase gene; PVHI, periventricular haemorrhagic infarction.

Table I: Characteristics of infants with an atypical and typical PVHI (*n*=62)

	n or median (range)	
	Atypical (*n*=17)	Typical (*n*=45)
Male	7	28
Female	10	17
PVHI		
Onset 6–96h postpartum	–	45
Antenatal onset (<6h postpartum)	9	–
Late onset (>96h postpartum)	8	–
Left-sided	10	24
Right-sided	6	19
Bilateral	1	2
No associated GMH-IVH	5	1
Associated GMH-IVH		
IVH grade I	1	1
IVH grade II	10	40
IVH grade III	1	3
PHVD	5	17
Surgical intervention for PHVD treatment	5 out of 5	10 out of 17
Subcutaneous reservoir	3 out of 5	7 out of 17
Subcutaneous reservoir and ventriculoperitoneal shunt	2 out of 5	3 out of 17
Gestational age (wks)[a]	30.6 (28.7–33.7)	29.6 (25.3–33.6)
Birthweight (g)	1170 (580–1990)	1200 (670–2210)
Small for gestational age (<10th centile)	4	3
Antenatal		
Antenatal corticosteroids[b]	11	22
Pre-eclampsia[c]	7	9
Chorioamnionitis[a,d]	0	14
Perinatal		
Apgar score at 1min	7 (3–9)	6 (0–10)
Apgar score at 5min	9 (7–9)	8 (3–10)
Apgar score (5min) <7[a]	0	9
Arterial umbilical cord pH	7.21 (7.04–7.31)	7.22 (6.82–7.38)
Emergency Caesarean section[a]	13	16
Respiratory problems		
Infant respiratory distress syndrome[a]	4	27
Pneumothorax	0	2
Ventilatory support (SIMV, HFO)[a,e]	3	27
Circulatory failure		
Inotropic drugs[f]	5	21
Patent ductus arteriosus[a,g]	1	14
Thrombocytopenia (<150×10^9/L)	9 out of 15	26 out of 39
Seizures[h]	3	11
Adverse neurological outcome[a,i]	13 out of 17	12 out of 42

[a]Statistically significant at $p<0.05$. [b]Administration of antenatal corticosteroids was defined as two doses of betamethasone more than 24 hours before birth. [c]Pre-eclampsia was defined as either pre-eclampsia or pregnancy-induced hypertension. [d]Chorioamnionitis was based on histopathological examination of the placenta. [e]In none of the infants with atypical, late-onset PVHI was ventilatory or circulatory support needed at the time of onset. [f]Circulatory failure was considered when there was need for fluid resuscitation and/or use of inotropic drugs because of haemodynamic instability during the first 24 hours after birth. [g]Patency of the ductus arteriosus was scored when treatment was necessary. [h]All infants were monitored by aEEG for at least 48 hours after diagnosis of PVHI and also routinely from admission onwards in those born before 30 weeks gestational age. Seizures were scored when electroencephalographically proven. [i]Adverse neurological outcome was defined as (expected) spastic unilateral CP or death due to extensive brain injury. aEEG, amplitude-integrated electroencephalography; GMH-IVH, germinal matrix haemorrhage – intraventricular haemorrhage; HFO, high-frequency oscillation; PHVD, post-haemorrhagic ventricular dilatation; PVHI, periventricular haemorrhagic infarction; SIMV, synchronized intermittent mandatory ventilation.

typical presentation, 16 had an early MRI (range 4–25d) and in 28 a TEA MRI was performed; no MRI was available in 17 infants.

Thrombophilia

Among the 17 Caucasian infants with atypical PVHI, a hetero-zygous FVL mutation was found in seven (41%); in one of these infants this was concomitant with a heterozygous prothrombin gene mutation (Table SI, supporting material online).

A polymorphism in the *MTHFR* gene was investigated in 16 out of 17 infants with atypical PVHI. Four infants were C677T heterozygous and two homozygous. There was heterozygosity for A1298C in one infant and homozygosity in three. Four infants were compound heterozygous. Thus 14 out of 16 infants had a polymorphism in the *MTHFR* gene.

None of the infants had elevated levels of lipoprotein(a) above 300mg/L or homocysteine levels above 10µmol/L. In one out of 17 infants, no thrombophilic factor was found.

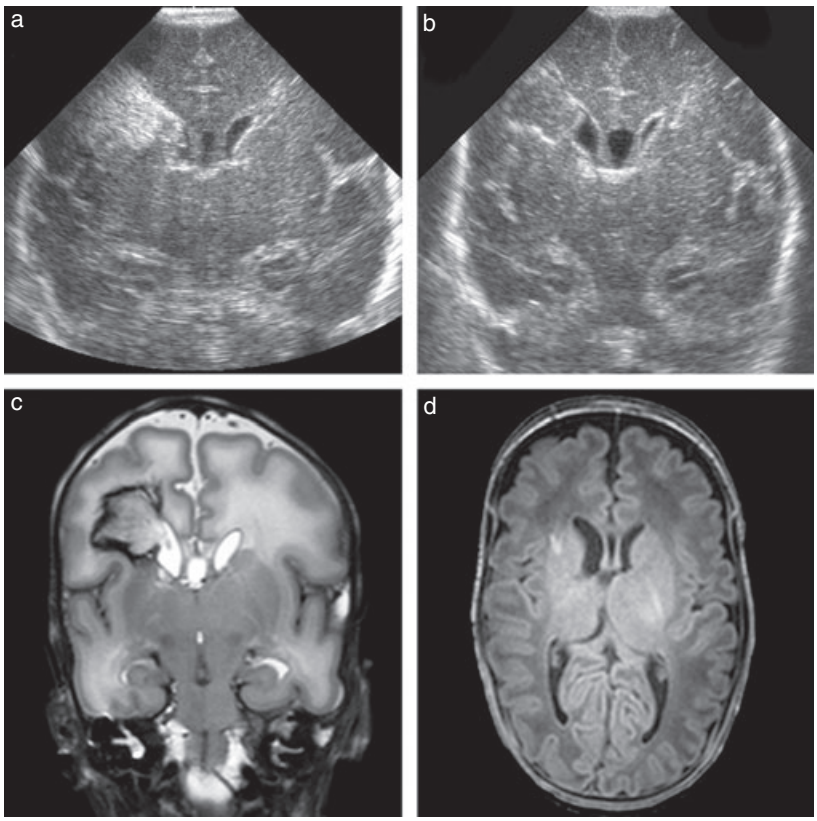

Figure 3: Male infant (case 1), born by emergency Caesarean section, gestational age 30 weeks and 5 days, birthweight 1170g (p16), Apgar scores 5 and 8 at 1 minute and 5 minutes respectively, umbilical cord pH 7.19. (a) First cranial ultrasound (cUS) immediately after birth shows large right-sided periventricular haemorrhagic infarction (PVHI) without an associated germinal matrix haemorrhage-intraventricular haemorrhage; (b) cUS and (c) magnetic resonance image (MRI) T2-weighted spin echo sequence on day 18 shows right-sided PVHI not communicating with the lateral ventricle and loss of white matter; (d) term-equivalent age MRI shows lack of myelination of the posterior limb of the internal capsule on the right side. At 16 months of corrected age, the infant showed spastic unilateral cerebral palsy on the left side. Coagulation profile: heterozygosity for factor V Leiden mutation and homozygosity for *MTHFR* C677T polymorphism; the thrombin and prothrombin time and lipoprotein(a) level at 22mg/L (0–300mg/L) were within normal limits. The homocysteine level at 3.8μmol/L (0–10μmol/L) was not elevated.

Two infants (cases 8 and 9) were diagnosed with a novel mutation in the *COL4A1* gene, as was reported previously.[17] *COL4A1* mutation analysis has not yet been performed in any of the other infants.

The mothers of the seven infants with a FVL mutation were tested and six of them were also diagnosed to have the mutation.

Neurological outcome

Thirteen of the 17 infants with atypical PVHI had a neurological examination at 18 to 22 months corrected age and 11 of these 13 were diagnosed with spastic unilateral CP. Asymmetry of the PLIC is predictive of the development of a spastic unilateral CP in two of the other four infants who are still younger than 18 months. In the infants with a FVL mutation and older than 18 months, five out of six developed spastic unilateral CP (Table SI, supporting material online). Manual ability of 9 infants older than 18 months was classified using the MACS: two were classified as level I, two as level II, four as level III, and one as level V.

In five infants with typical PVHI, care was withdrawn because of extensive brain injury and one died owing to necrotising enterocolitis. Twenty-five infants with a typical PVHI were older than 18 months corrected age and in three of them a diagnosis of spastic unilateral CP was made. They were all classified at level I using the MACS. Fourteen infants were younger than 18 months corrected age and 11 of these had a TEA MRI showing an asymmetry of the PLIC in four of them. Therefore, seven infants (19%) had or were expected to develop spastic unilateral CP.

DISCUSSION

Risk factors for PVHI in preterm neonates have been studied extensively in recent decades.[6–9] Several research groups have examined thrombophilic mutations in preterm infants with GMH-IVH.[14–16,22] Among 62 preterm infants with a PVHI, we found 17 (27%) with an atypical presentation, which was the reason to investigate thrombophilic mutations. Seven of them were heterozygous for the FVL mutation, suggesting an association between this most common hereditary hypercoagulable state and development of atypical PVHI.

Mothers with a child with a FVL mutation were tested for thrombophilic mutations as well and all but one were found to

have this mutation. The mother of case 4 developed a pulmonary embolism a few days after delivery. The sister of the mother of case 1 suffered from a myocardial infarction at 27 years of age. A significant contribution of maternal thrombophilia to a higher prevalence of perinatal arterial ischaemic stroke has recently been reported.[23]

All but one of the infants with a FVL mutation developed spastic unilateral CP, which could be explained by the size and site of the lesion. This is in agreement with a study by Mercuri et al.,[24] who suggested that infants with perinatal arterial ischaemic stroke and a heterozygosity for FVL mutation were more likely to develop spastic unilateral CP than those without this mutation. To the best of our knowledge, there has been no previous study of the neurological outcome of PVHI in relation to the presence of a thrombophilic mutation or an atypical development of PVHI. There are, however, increasing data showing a genetic cause of CP.[25,26]

Most known risk factors in the pathogenesis of PVHI are associated with systemic and cerebral haemodynamic instability in a critically ill preterm infant.[6] These include emergency Caesarean section, low Apgar scores, patent ductus arteriosus, acidosis, respiratory distress syndrome, vasopressor and respiratory support, pulmonary haemorrhage, and pneumothorax.[6–9] None of these risk factors, except for emergency Caesarean section, were seen in the infants with an atypical presentation preceding PVHI.

PVHI is almost invariably associated with a large GMH-IVH on the ipsilateral side[4,5] but this was not seen in four of the seven infants with a FVL mutation who either had a small associated GMH-IVH or no associated haemorrhage at all (Table SI, supporting material online). The absence of a large associated haemorrhage or an atypical presentation, such as antenatal onset or late presentation in the absence of known risk factors, were reasons to perform additional investigations in the 17 infants diagnosed to have atypical PVHI. This led to the finding of an associated thrombophilia in the seven infants reported here, and to a diagnosis of *COL4A1* mutation in two infants reported previously (case 8 and 9).[17] In one infant with a late PVHI in the context of a severe illness, there was a diagnosis of extensive cerebrosinovenous thrombosis (CSVT) comparable with a case reported by Ramenghi et al.[27] In preterm infants with CSVT, however, the parenchymal lesions tend to be bilateral and are seen in the presence of an IVH.[27]

In the FVL variant, a point mutation in the factor V gene results in the replacement of the 506th amino acid arginine to glutamine in one of the activated protein C cleavage sites. Activated protein C specifically cleaves peptide bonds in activated factor V, resulting in inhibition of the coagulation pathway. The FVL mutation thus results in resistance of factor V to inactivation by activated protein C and consequently in hypercoagulability.[10,11] The prothrombin gene mutation involves a transition from guanine to adenine at nucleotide position 20 210 and is associated with slightly increased plasma levels of prothrombin and a mild hypercoagulable state.[11] It is well established that hypercoagulability increases the risk of venous thrombosis in childhood.[10,11] Since venous congestion is involved in the pathogenesis of PVHI, hypercoagulability might have contributed to the development of PVHI.

The prothrombotic contribution of polymorphisms in the enzyme MTHFR is different from the FVL and prothrombin mutation. In a Dutch population-based study, 10% were found to be homozygous and 44% heterozygous for the C677T polymorphism.[28] MTHFR is important in catalysing 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is the major circulating form of folate. Folate is involved in methylation of homocysteine. The C677T and A1298C polymorphisms of the *MTHFR* gene are associated with decreased activity of this enzyme and result in elevated plasma homocysteine levels. However, an elevated plasma level of homocysteine (>10μmol/L) was not found in any of the infants in our study. Although the role of this mutation in the genesis of neonatal stroke is not yet clear, a possible effect in neonatal cerebral thromboembolic disease cannot be excluded.[29]

In our study, levels of protein C, protein S, and antithrombin were not tested in all infants. It can therefore not be excluded that the presence of transiently reduced activities of these coagulation factors may play a role in the development of PVHI. In newborn infants, however, levels of these anticoagulants are significantly lower than in older children and adults. Whether these transient physiologically reduced levels play a role in the pathogenesis of cerebral thrombovascular disease is still unclear.[30] Laboratory investigations of these anticoagulants are difficult to interpret before the age of 6 months. Inherited deficiencies in protein C, protein S, and antithrombin have a low prevalence, but in several studies a positive correlation of quantitative or qualitative protein C deficiency with neonatal and childhood ischaemic or haemorrhagic stroke has been reported.[23,25,29,30] In one of our infants (case 4), slightly lower activity of protein C was present in the neonatal period as well as at the age of 4 years. In another infant (case 2), a reduced level of antithrombin was reported in the acute phase.

Elevated lipoprotein(a) is a recognized predisposing factor for embolism in children and newborn infants based on the competition with plasminogen for fibrin binding and thus impaired fibrinolysis.[31] Lipoprotein(a) levels were tested in all infants, with the exception of case 5, but an elevated level (>300mg/L) was not found in any of them.

In 2005, Gould et al.[18] reported the presence of a mutation in a basement membrane protein, procollagen type IV α 1, causing vascular defects. This can lead to perinatal cerebral haemorrhage and can predispose to porencephaly. In two infants (cases 9 and 10) with antenatal development of porencephaly, we found a novel mutation in this *COL4A1* gene as previously reported.[17]

The association between PVHI and FVL and prothrombin G20210A mutation is consistent with the findings of Petäjä et al.[16] In 22 infants with neonatal IVH grade II to IV they found significantly more FVL mutations compared with 29 very-low-birthweight infants in a control group. They also emphasized the case of an infant born at term with a PVHI and FVL mutation. Because of the low risk of IVH near term,

they suggested that FVL mutation could be a risk factor for PVHI. Komlósi et al.[14] showed an increased prevalence of FVL mutation in 60 preterm infants (<36wks gestational age) with grade I IVH in comparison with 63 matched controls. Gopel et al.[15] investigated prothrombotic mutations in a cohort of 318 very-low-birthweight infants with IVH grade I to IV. They proposed a protective effect of the FVL or prothrombin G20210A mutation against the extension of the bleeding from grade I to grade II stage or more. However, the frequency of IVH grade IV remained the same between very-low-birthweight infants with these prothrombotic mutations and infants without. In contrast, in a large case–control study of 586 infants, Hartel et al.[22] found no association between prothrombotic gene variants, including FVL and prothrombin mutation, and IVH grade I to IV.

The data presented here are different from previous data, as the infants in this study had an atypical presentation, which was the reason for performing the mutation analysis. Heterozygosity for FVL mutation was present in seven out of 17, which is much higher than the prevalence in the Dutch population (3–5%)[32] and the Finnish population (4%).[16] Not having performed mutation analysis in all preterm infants with a PVHI is a limitation of this study. A prospective study is required to assess the true prevalence of thrombophilia in preterm infants with typical PVHI, but we expect the prevalence to be low, in agreement with previous studies.

COL4A1 mutation analysis was also only performed in the infants with antenatally acquired porencephaly and, in view of a recent observation in twins born at 24 weeks of gestation,[33] may be more common in both typical and atypical PVHI. Another limitation of this study is that only seven of 17 infants with an atypical presentation had an early MRI with MR venography to diagnose or exclude the presence of CSVT.

The presence of a prothrombotic gene mutation in neonates with PVHI has no immediate therapeutic consequences, except when a CSVT is diagnosed and anticoagulation therapy with low-molecular-weight heparin may be considered. According to recent guidelines, however, this is not recommended in the presence of a parenchymal haemorrhage.[34,35] Nevertheless, demonstrating the presence of thrombophilic mutations in infants as well as in their mothers is important. They may be predisposed to higher risks of thrombosis,[10] and counselling on avoidable circumstantial risk factors (e.g. contraceptives and long-distance travelling) and the impact of lifestyle factors (e.g. obesity and immobility) is important. There is also an increased risk of complications in a future pregnancy or in the postpartum period, as was seen in our study where one mother had a pulmonary embolism a few days after delivery. In the case of *COL4A1* gene mutations, there are no therapeutic options available but there is a risk of recurrence when this presents as an autosomal dominant familial porencephaly. Conscientious counselling in future pregnancy is therefore of utmost relevance.

CONCLUSION

In half of the infants in this study with atypical PVHI, a genetic mutation was found, especially heterozygosity for FVL.

Testing for mutations or polymorphisms in thrombophilic and collagen genes in newborn infants with an atypical presentation of PVHI should be considered. Additional information can be obtained by testing the mother.

ONLINE MATERIAL/SUPPORTING INFORMATION

Additional material and supporting information for this paper may be found online.

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