# Factor V Leiden and genetic defects of thrombophilia in childhood porencephaly

Otfried Debus, Hans Georg Koch, Gerhard Kurlemann, Ronald Sträter, Heinrich Vielhaber, Peter Weber, Ulrike Nowak-Göttl

**Abstract**

***Aims*—To determine to what extent the Arg$^{506}$ to Gln point mutation in the factor V gene and further genetic factors of thrombophilia affect the risk of porencephaly in neonates and infants.**

***Methods*—The Arg$^{506}$ to Gln mutation, factor V, protein C, protein S, antithrombin, antiphospholipid antibodies and lipoprotein (a) (Lp(a)) were retrospectively measured in neonates and children with porencephaly (n=24).**

***Results*—Genetic risk factors for thrombophilia were diagnosed in 16 of these 24 patients: heterozygous factor V Leiden (n=3); protein C deficiency type I (n=6); increased Lp (a) (n=3); and protein S type I deficiency (n=1). Three of the 16 infants had two genetic risk factors of thrombophilia: factor V Leiden mutation combined with increased familial Lp (a) was found in two, and factor V Leiden mutation with protein S deficiency type I in one.**

***Conclusions*—The findings indicate that deficiencies in the protein C anticoagulant pathway have an important role in the aetiology of congenital porencephaly.**

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Keywords: porencephaly; factor V Leiden mutation; protein C; lipoprotein (a)

Porencephaly and hydranencephaly are fluid filled cavities in the cerebral hemispheres, which may communicate with the spaces containing cerebrospinal fluid: they are partial or subtotal defects of the developing cerebral hemispheres. Their reported overall prevalence is 2.5% in children with infantile brain damage.[1] The aetiology and pathogenesis of porencephaly and hydranencephaly are still controversial. Yakovlev and Wadsworth described porencephaly as a malformation resulting from incomplete closure of the brain vesicle,[2] but the belief that both porencephaly and hydranencephaly are residuals resulting from destruction of brain tissue from a failure in the carotid circulation is more widely accepted.[3–12] Various noxious events during the fetal period are involved, including inflammation, haemorrhage, or exposure to vasoactive drugs.[3–15]

Dahlbäck *et al* recently described in vitro resistance to the anticoagulant response of activated protein C (APC),[16] in most cases associated with the Arg$^{506}$ to Gln point mutation in the factor V gene in thrombophilic patients.[17] To determine to what extent this common gene mutation and further genetic factors of familial thrombophilia affect the risk of porencephaly in the fetus and infancy, its occurrence was retrospectively investigated in a population of children with congenital cystic malformations.

## Methods

Twenty four children aged 0 to 18 years treated for impairments due to perinatally acquired porencephalic cysts during the past 10 years were enrolled in the study. Computed tomography scans, magnetic resonance (MR) imaging, or transcranial Doppler ultrasonography were performed in all children to confirm the diagnosis of porencephaly.

Clinical, neurodevelopmental, and electroencephalographic (EEG) examinations were also carried out at the onset of symptoms and in the follow up period in the children investigated.

Blood samples for coagulation studies were drawn from a peripheral vein into pre-marked 3 ml plastic tubes (citrate 3.8%/blood 1:10; Saarstedt), immediately placed on iced water and centrifuged at 4°C at 3000 × *g* for 20 minutes. Platelet poor plasma was snap-frozen and stored in plastic tubes at -70°C. The response to activated protein C (Chromogenix, Mölndal, Sweden), the factor V Leiden mutation (DNA prepared from EDTA blood), factor V (factor V deficient plasma: Instrumentation Laboratory, Munich, Germany), protein C (chromogenic substrate S 2366: Chromogenix, Mölndal, Sweden), protein S (based on the prolongation of a prothrombin time (IL Test): Instrumentation Laboratory, Munich, Germany), antithrombin (chromogenic substrate S 2765: Chromogenix, Mölndal, Sweden), antiphospholipid antibodies (IgM/IgG: Stago, Asnières-sur Seine, France) and lipoprotein (a) concentration (Lp (a): Chromogenix, Mölndal, Sweden) were measured, as described before.[18 19] Free and total protein S, and protein C antigens were also measured using an enzyme linked immunoabsorbent assay (ELISA) technique (Asserachrom, Stago: Asnières-sur-Seine, France).

All children were investigated at the first clinical presentation and were re-investigated when APC and the factor V Leiden mutation were routinely investigated. Family studies (parents, and if available, grandparents, brothers, and sisters) were also performed for all children affected. Furthermore, possible thromboembolic events (deep venous thrombosis, myocardial infarction, or stroke) and

**Department of Paediatrics, University Hospital Munster, Germany**
O Debus
H G Koch
G Kurlemann
R Sträter
H Vielhaber
P Weber
U Nowak-Göttl

Correspondence to:
Dr Ulrike Nowak-Göttl, Westfälische Wilhelms-Universität, Paediatric Haematology and Oncology, Albert-Schweitzer- Str. 33, D - 48149 Münster, Germany.

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previous histories of fetal wastage were carefully evaluated in all families.

In infants and children with suspected familial thrombophilia the final diagnosis was made when DNA based assays confirmed the diagnosis ($Arg^{506}$ to Gln mutation of the factor V gene), or when repeatedly measured plasma concentrations were outside the age appropriate reference range, and at least one family member carried the same genetically determined coagulation defect.[20–22]

## Results

The patients' characteristics are shown in table 1. Seizures, often presenting as hemiconvulsions, were the principal symptoms in neonates and infants up to 3 months of age; convulsions or contralateral hemiparesis were the initial symptoms in infants with porencephaly and onset at less than 4 months of age. In most cases an early onset immediately after birth or within the first three months of life was reported.

In most cases porencephaly occurred in the left hemisphere (n=14). Only few porencephalic cysts were located in the right hemisphere (n=4). Polycystic porencephaly (n=5) and hydranencephaly (n=1) were also reported in the children investigated.

In neonates and infants under 6 months of age the initial diagnosis was documented by ultrasonography (n=22), confirmed by computed tomography (n=12), MRI (n=9) or necropsy (n=1) in the follow up period. In children >1 year of age, presenting with focal seizures or hemiparesis, the diagnosis was primarily confirmed by computed tomogram (n=1) and MRI (n=1).

In 16 out of 24 infants and children with porencephaly, genetic risk factors for familial thrombophilia were diagnosed: heterozygous factor V Leiden (n=3); protein C deficiency type I (n=6); increased Lp (a) (n=3); and protein S type I deficiency (n= 1). In addition, three of the 16 infants had two genetic risk factors of thrombophilia: factor V Leiden mutation combined with increased familial Lp (a) was found in two, and factor V Leiden mutation with protein S deficiency type I in one infant. Neither antithrombin nor factor V deficiency and no increased antiphospholipid antibodies were found in the population studied.

A positive family history of thrombosis was found in five out of 24 families studied. In addition, maternal risk factors during pregnancy—that is, viral or bacterial infections, pre-eclampsia, placental dysfunction or fetal

*Table 1 Sex, age at onset, initial symptoms, cystic location, genetic risk factors, age at final diagnosis, outcome and family history in children with porencephaly*

| *Sex* | *Onset* | *Initial symptoms* | *Porencephalic location* | *Genetic risk factor* | *Age at final diagnosis* | *Outcome* | *Family history* |
|---|---|---|---|---|---|---|---|
| M | 1 day | Focal seizures | L middle cerebral artery | F V Leiden / Lp (a): 120 mg/dl | 2 years | Lennox | + |
| M | 6 weeks | Seizures | L middle cerebral artery | F V Leiden | 4 years | Lennox | − |
| M | Birth | Focal seizures | R middle cerebral artery | Protein S type I free ag: 8%; total: 10% | 4 weeks | Blindness death | + |
| F | Birth | Hypotonia seizures | L middle cerebral artery | Protein C type I ac: 25%; ag 20% | S years | Lennox | − |
| M | 3 years | Macrocephaly L hemiparesis | R middle cerebral artery R posterior artery | Lp (a): 60 mg/dl | 16 years | L hemiparesis | − |
| M | 8 weeks | Hypotonia seizures | R/L middle cerebral artery | Lp (a): 70 mg/dl | 7 years | Tetraparesis/ retardation | − |
| M | 8 weeks | Seizures | R middle cerebral artery | Protein C type I ac: 40%; ag 45% | 15 years | L hemiparesis | − |
| M | 8 weeks | Focal seizures | L middle cerebral artery | | 14 years | L hemiparesis | − |
| M | 5 months | Seizures L hemiparesis | R middle cerebral artery | F V Leiden Lp (a): 145 mg/dl | 18 years | L hemiparesis | + |
| M | Birth | Seizures | L middle cerebral artery L posterior artery | F V Leiden Protein S type I free ag: 15%, total: 20% | 3 years | R hemiparesis Severe retardation | + |
| F | 2 years | Seizures | L middle cerebral artery | Protein C type I ac: 22%; ag: 30% | 4 years | No handicap | − |
| M | 4 weeks | Focal seizures | L middle cerebral artery | F V Leiden | 8 years | R hemiparesis | − |
| M | 6 weeks | Focal seizures | L middle cerebral artery | | 10 years | R hemiparesis: mild | − |
| F | 3 months | Focal seizures | L middle cerebral artery | Lp (a): 90 mg/dl | 16 years | R hemiparesis: mild | − |
| M | 4 months | L hemiparesis Seizures | R middle cerebral artery | | 6 years | L hemiparesis | − |
| M | 4 months | R hemiparesis | L middle cerebral artery L posterior artery | | 10 years | R hemiparesis: mild | − |
| F | 5 months | Tetraparesis Seizures | R/L middle cerebral artery | F V Leiden | 5 years | Tetraparesis Blindness/ retardation | − |
| F | 3 months | Focal seizures | L middle cerebral artery L anterior artery | Protein C type I ac: 15%; ag: 22% | 2 years | R hemiparesis Retardation | + |
| F | 8 weeks | Macrocephaly Seizures | L posterior artery | | 13 years | No handicap | − |
| F | Birth | Focal seizures | L middle cerebral artery | Protein C type I ac: 51%; ag: 50% | 12 years | R hemiparesis Retardation | − |
| M | 4 months | R hemiparesis Seizures | L middle cerebral artery | | 4 years | R hemiparesis Retardation | − |
| M | 3 months | Focal seizures | L middle cerebral artery | | 18 years | R hemiparesis Blindness Retardation | − |
| F | Birth | Focal seizures | L middle cerebral artery | Protein C type I ac: 22%; ag 20% | 4 years | R hemiparesis | − |
| F | 4 months | Seizures R hemiparesis | L middle cerebral artery | | 14 years | R hemiparesis Retardation | − |

M=male; F=female; L=left; R=right; Lp (a)=lipoprotein (a); ac=activity; ag: antigen.

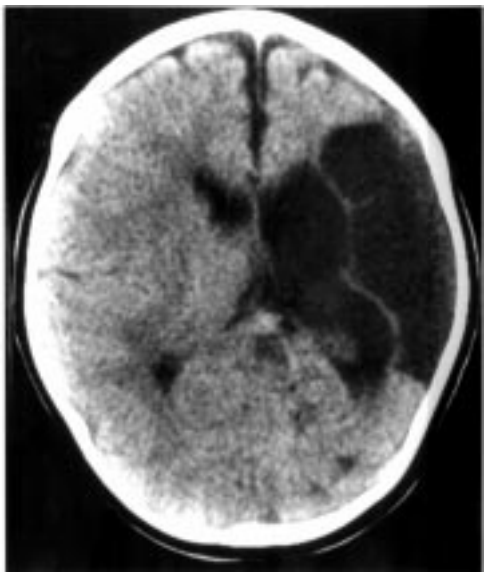

*Figure 1 Computed tomogram showing intrauterine porencephalic cystic formation (two cavities) in a boy heterozygous for the factor V Leiden mutation combined with familial increased lipoprotein (a) values.*

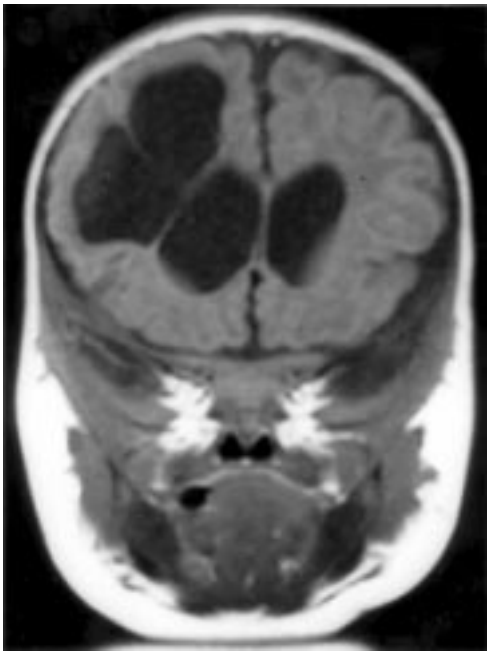

*Figure 2 Magnetic resonance imaging in a boy with heterozygous factor V Leiden mutation showing porencephalic cyst in the right temporal area.*

bradyarrhythmia—were documented in nine of the 24 mothers investigated.

Figures 1 to 3 show cystic malformation in the area of the left middle cerebral artery (fig 1), right temporal (fig 2), and left parieto-temporal (fig 3) in a child heterozygous (+/-) for the common factor V mutation combined with familiarly increased lipoprotein (a) (fig 1), the common factor V (+/-) mutation (fig 2), and one child without genetic risk factors for familial thrombophilia (fig 3).

One infant died at the age of 6 months. Mental retardation, tetraplegia, and blindness was found in seven of the remaining 23 patients studied. Contralateral hemiplegia was also reported in 16 of the 23 cases. Lennox-Gastaut syndrome, diagnosed clinically in combination with slow psychomotor development or mental retardation, and confirmed by EEG criteria (slow (<2.5 Hz), spike-wave activities), was found in three of the children investigated.[23] Only five of the 23 children showed mild or no handicap.

Only three of the neonates investigated who had had additional deep venous thrombosis at the onset of the disease were treated with low dose unfractionated heparin over a period of three months. No further anticoagulation was given. None of the children enrolled in this retrospective study developed deep venous thrombosis or recurrent stroke in the follow up period.

## Discussion

The pathology of neonatal cerebral infarction is unknown, and only few necropsy data are available because the babies affected rarely die.[24] Besides porencephaly resulting from incomplete closure of the brain vesicle,[2] trauma, infectious diseases, vasoactive drugs (such as cocaine), some authors suggest an embolic or thrombotic origin in neonates and infants with stroke.[3–15 25] A high incidence of peripartal asphyxia is also reported[26 27]; others have implicated birth trauma.[28]

To underline the multifactorial pathology of perinatal/neonatal stroke this study focused on the genetic risks of thromboembolism. In this retrospective analysis we found a high prevalence of genetic risk factors (66%) for familial thrombophilia in neonates and infants with porencephalic cystic lesions.

In childhood stroke deficiencies of physiological anticoagulants such as antithrombin, protein C, or protein S have rarely been reported.[29–35] Defects of the protein C anticoagulant system associated with thrombophilia

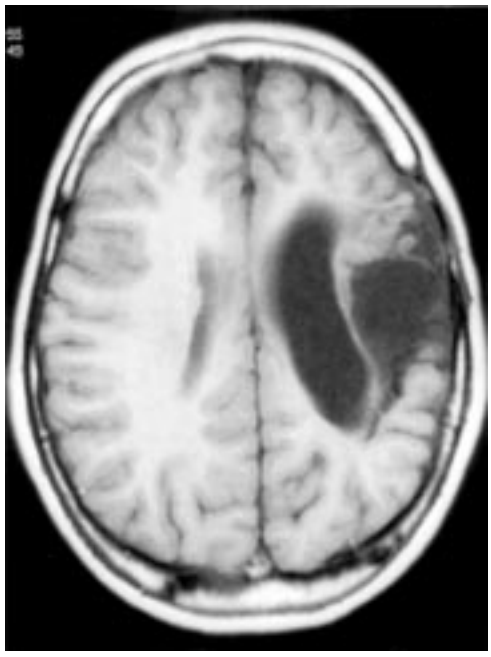

*Figure 3 Magnetic resonance imaging in a boy with no genetic risk factor for familial thrombophilia: left parieto-temporal porencephaly is shown.*

are usually transmitted with an autosomal dominant pattern of inheritance. The prevalence of protein C deficiency is 3.2% in unselected patients with venous thrombosis and 3.8% in selected patients. In the general population extrapolated data obtained for cohorts of thrombotic patients indicate a prevalence of between 1 in 16 000 and 1 in 36 000. A much higher prevalence was found in healthy subjects, with a rate of 1 in 200 to 1 in 300. The prevalence of protein S deficiency is similar to that of protein C deficiency: 2.2% for unselected patients with venous thrombosis and 3 % for selected patients. However, there are no data on the prevalence of protein S in the general population. Bearing in mind the high frequency of the factor V Leiden mutation of between 4 and 10% in Europe and North America, the prevalence of APC resistance is 21% in unselected patients with primary thromboembolism and 22% in selected patients.[36]

Similar to childhood stroke,[37] the factor V Leiden mutation and protein C deficiency have an important role in the aetiology of porencephalic perinatal cysts. Six out of 24 children enrolled in this study had the $Arg^{506}$ to Gln mutation in the factor V gene.

Strokes in young adults were attributed to antithrombin, protein C, or protein S deficiency in 17 of 54 cases investigated.[38] In our study besides the $Arg^{506}$ to Gln mutation in the factor V gene, children with porencephaly and inherited thrombophilia showed protein C deficiency type I (n=6), one infant showed protein S deficiency type I, and five children had familiarly increased Lp (a), which has recently been found to be a risk factor for stroke in child and adult patients <70 years of age.[39 40] However, bearing in mind the large number of asymptomatic individuals with deficiencies in the protein C pathway, intrauterine viral/bacterial infections, placental dysfunction, or arrhythmia should be considered additional possible factors triggering early thromboembolism in the neonates, infants, and children investigated.

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