

» Design of a prospective neonatal cohort study of homozygous and double heterozygous Factor V Leiden and Factor II G20210A

Abstract. Background: Factor V Leiden (FVL) and Factor II (FII) G20210A represent common risk factors for thromboembolic (TE) events. In children, both venous and arterial TE-events have been associated with the presence of FVL and FII G20210A. In most heterozygous children with TE-events other prothrombotic factors can usually be identified. Case reports of children with homozygous FVL, including 3 patients described here, suggest that this genotype may convey a particularly high risk. However, prospective data about the type and frequency of TE-events in such children are lacking. **Study design:** We have initiated a prospective neonatal cohort study for the homozygous and double heterozygous genotypes for FVL and FII G20210A. The probands and the heterozygous controls are identified by neonatal screening that involves >98% of the children born in Berlin and are followed up in a special out-patient clinic to document details of the clinical history, developmental parameters and the occurrence of TE-events. **Conclusions:** This study will provide controlled and unbiased information about the clinical significance of the homozygous and double heterozygous genotypes of these mutations.

Key words: Thrombosis in children – Factor V Leiden – Factor II G20210A

Design einer prospektiven neonatalen Kohortenstudie zum homozygoten oder doppelt heterozygoten Genotyp für die Faktor V Leiden und die Faktor II G20210A Mutationen. Hintergrund: Die Faktor V Leiden (FVL) und die FII G20210A Mutationen stellen bekannte Risikofaktoren für thromboembolische (TE-) Ereignisse dar. Im Kindesalter sind sowohl venöse als auch arterielle TE-Ereignisse mit dem Vorkommen der FVL oder der FII G20210A Mutation assoziiert. Bei heterozygoten Kindern mit TE-Ereignissen können meist weitere prothrombotische Faktoren identifiziert werden. Mehrere Fallberichte lassen für Kinder mit homozygotem Genotyp für FVL ein höheres Risiko vermuten. Prospektive Daten über Häufigkeit und Typ von TE-Ereignissen bei solchen Kindern liegen jedoch bislang nicht vor. **Studiendesign:** Wir initiierten eine prospektive neonatale Kohortenstudie zum homozygoten und doppelt heterozygoten Genotyp für die FVL und die FII G20210A Mutationen. Die Probanden und die heterozygoten Kontrollen werden im Rahmen des neonatalen Screening identifiziert, welches >98% der Neugebore-

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nen in Berlin umfasst. Die Follow-up-Untersuchungen beinhalten die Dokumentation der Anamnese, des entwicklungsneurologischen und allgemein-pädiatrischen Untersuchungsbefundes und des Auftretens von TE-Ereignissen. **Schlussfolgerung:** Diese Studie wird kontrollierte und prospektive Informationen zur klinischen Bedeutung des homozygoten und des doppelt heterozygoten Genotyps für diese Mutationen liefern.

Schlüsselwörter: Thrombose bei Kindern – Faktor V Leiden – Faktor II G20210A

Introduction

Thromboembolic (TE) events during childhood are rare, but the first year of life and the time after puberty are periods of particular risk for thrombosis [1]. Factor V Leiden (FVL) and the Factor II G20210A (FII) mutations are the most common causes of hereditary thrombophilia in adults [2,18,22] as well as in children [16,13,23]. In contrast to adulthood, in children FVL and the FII mutation are associated with venous and also with arterial thrombotic events involving a variety of different organs [3–5,7–12,14,16,17,19,21,24]. In most heterozygous children with TE-events additional, genetic or non-genetic risk factors can be identified. In the Leiden thrombophilia study that analyses a cohort of adult patients with a history of deep venous thrombosis, the first TE-event of patients with a homozygous FVL genotype tended to occur during early adulthood [20]. This indicated that homozygous FVL leads to a high risk of deep venous thrombosis and suggested that thrombosis in this group may not likely occur before adulthood. In contrast, numerous reports including those patients described below have shown homozygous FVL to be associated with venous and arterial TE-events in children [7–9,12,15,17,21] and even with the risk of preterm birth [6].

As there are no prospective data about the type and frequency of TE-events in children with the homozygous or double heterozygous genotypes for the FVL and the FII mutations we have initiated a prospective pediatric cohort study of probands with these genotypes.

Case reports

The first child was a previously healthy 11-year-old girl, who suddenly developed a left sided hemiparesis including the leg, arm, face and tongue. Magnetic resonance tomography (MRT) of the brain showed a right-sided lesion near the

capsula interna compatible with an ischemic stroke. Intracardial thrombi or a persistent foramen ovale were excluded by echocardiography. Doppler sonography showed normal extracranial arteries. DNA analysis revealed the homozygous genotype for FVL. Protein C, protein S and antithrombin activities and plasminogen and fibrinogen levels were normal. Antiphospholipid antibodies (APLA) were not detected. Serological tests indicated a past infection with *Borrelia burgdorferi*. *Borrelia burgdorferi*-IgG and an elevated cell count in the cerebro-spinal fluid suggested the presence of neuroborreliosis. Both parents were heterozygous for the FVL mutation, but there was no family history for TE-events.

The second patient was a 7-month-old boy, who was presented at the emergency department because of acute otitis media. The mobility of his right arm had been noticed by the parents to be reduced during the previous 5 months. The muscle tone and the reflexes of the right arm were increased, while sensitivity appeared to be normal. An MRT of the brain revealed a cystic lesion in the left parietal lobe compatible with an old ischemic stroke by thrombosis of the A. cerebri media (Fig. 1). Echocardiography was normal. Assays for protein C, protein S, antithrombin and APLA showed no abnormalities. DNA analysis identified the homozygous genotype for FVL. The parents of this patient were 2nd degree

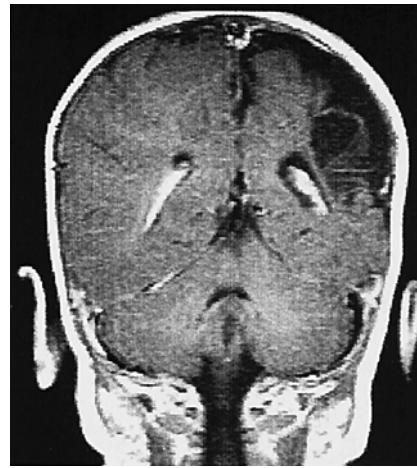
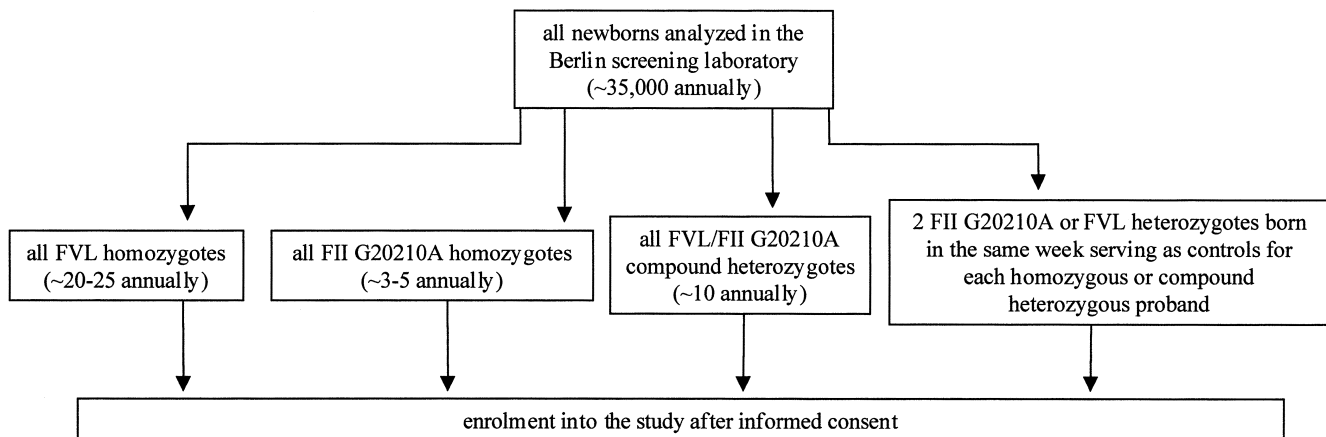


Fig. 1 MRT scan of the brain of patient # 2 revealing a cystic lesion in the left parietal lobe.

relatives and were heterozygous carriers of the FVL mutation. There was no family history of TE-events.

The third patient was a 4-year-old previously healthy girl, who was admitted with a non-febrile seizure that developed into a status epilepticus lasting for eight weeks despite most



First visit

1. documentation of the family history and the neonatal data
2. clinical examination
3. ultrasonography of kidneys and brain
4. genotyping of the family

3 monthly follow up visit

1. history
2. clinical examination and developmental status
3. ultrasonography of the brain

1 year follow up visit

- 3 monthly follow up plus measurement of protein C, protein S, antithrombin, lp(a), plasma homocystein, APLA

Fig. 2 Study design of the prospective neonatal cohort study of homozygous and double heterozygous Factor V Leiden and Factor II G20210A mutations (FVL = Factor V Leiden; lp(a) = lipoprotein a; APLA = antiphospholipid antibodies).

intensive therapeutic interventions. The patient had to be mechanically ventilated for four weeks. Following this episode, there was no fixation of gaze, no verbal response and the patient showed no localisation of pain. Furthermore, there were frequent generalized seizures.

Intracranial abnormalities could not be detected either by MRT, computer-tomography (CT), or MR angiography. Electroencephalography identified no single focus of hypersynchronous activity. There was no evidence for an infectious or metabolic origin of the seizures. DNA analysis revealed a homozygous genotype for FVL. In this unusual patient, neither vascular nor non-vascular causes for the status epilepticus could be unequivocally identified. The presence of a homozygous genotype for FVL led us to hypothesize thrombosis of small vessels that were not detectable by radio- or MR-imaging. There was no family history of TE-events, although the mother and the 2-year-old brother of the propositus also displayed a homozygous genotype for the FVL mutation.

Study design

We have initiated a prospective neonatal cohort study for the homozygous and the double heterozygous genotypes for FVL and FII G20210A, which has been approved by the ethics committee of the Charité Medical Center, Humboldt-University of Berlin.

The probands are identified by neonatal screening that involves >98% of the neonates born in Berlin (Fig. 2). In accordance with recently updated guidelines for neonatal screening issued by the German Society for Pediatrics, in Berlin the parents' written consent for the general screening and for testing in the context of this study is now obtained before blood sampling.

At an annual birth rate of ~35000 and reported gene frequencies of 0.025 (FVL) and 0.01 (FII mutation) we expect to identify 20–25 FVL and 3–5 FII mutation homozygotes and ~10 double heterozygous infants per year, respectively.

Parents of newborns with the homozygous or the double heterozygous genotypes for FVL and the FII mutation are contacted and, following informed consent by the parents, the children are enrolled into the study. Homozygous or double heterozygous siblings are also enrolled. For each proband two sex-matched heterozygous infants born in the same week are included into the study to serve as controls. Families living outside Berlin precluding regular follow up are excluded from the study. The occurrence of a severe thromboembolic event and loss to follow up define end points of the study.

An early detection of an increased incidence of severe TE-events in homozygous or double heterozygous probands necessitating the development of prophylactic strategies represents a termination criterium of the study.

During the primary visit the personal and family histories are documented with particular attention to previous thrombotic events, premature births, and spontaneous abortions. The children are examined with particular attention to their vascular status and ultrasonography of brain and kidneys is

performed. The FVL and FII genotypes of the parents of the heterozygous controls are determined. Follow-up visits are performed at 3-monthly intervals during the first year of life and at 6-monthly intervals during second and third year of life. At these visits particular attention is paid to the developmental parameters of the children and serial ultrasonography of the brain is performed until the anterior fontanelle has closed. Other hypercoagulable states are excluded at 1 year of age (Fig. 3).

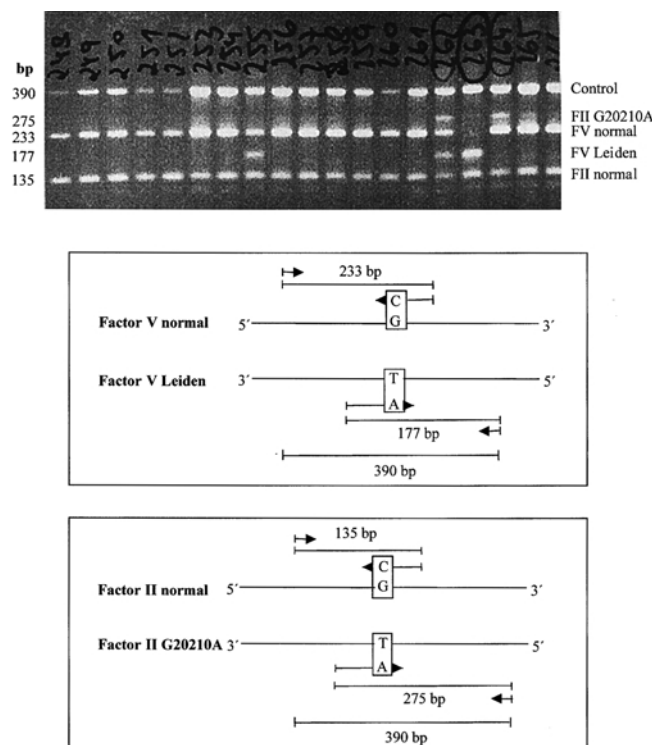


Fig. 3 Allele specific multiplex PCR for the FVL and FII G20210A mutations. Methods: 3 mm discs are punched out of the screening filter cards and are directly used for PCR analysis in a 96 well plate in a PTC-100 PCR cyclor (MJ-Research). In a first step, the samples are immersed in PCR buffer including the primers and are subjected to 3 pre-cycles (3 min 95 °C, 3 min 60 °C). Subsequently the Taq polymerase is added and the allele specific PCR with 40 cycles started (30 s 95 °C; 90 s 60,5 °C; 30 s 72 °C). This allele-specific PCR is directed to identify the normal Gs at codon 506 of the FV gene and at position 20210 of the FII gene or the FVL and the 20210 G→A mutations, respectively. The choice of the primers resulted in the generation of five different specific fragments of 390 bp (positive control for the PCR reaction), 275 bp (FII G20210A), 233 bp (FV normal), 177 bp (FVL), and 135 bp (FII normal). The arrows indicate the positions of the primers and the bars the size of the allele specific PCR products. The band pattern allows the definition of the genotype (sample #255: FVL heterozygous; #262: FII G20210A/FVL double heterozygous; #263: FVL homozygous; #264 and #265: FII G20210A heterozygous; all others: normal genotype).

Conclusion

The unselected identification of newborns with the homozygous and double heterozygous genotypes for the factor FVL and the FII mutation will provide controlled and prospective

information about the type and frequency of thromboembolic events in these groups of individuals.

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