

## FACTOR V LEIDEN AND PROTHROMBIN GENE 20210A VARIANT IN NEONATAL THROMBOEMBOLISM AND IN HEALTHY NEONATES AND ADULTS: A Study in a Single Center

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□ *This study was conducted to identify the prevalence of FV1691A and PT20210A mutations in neonates with symptomatic thromboembolism and in healthy neonates and adults. A review of 137 healthy neonates, 368 healthy adults, and 9 neonates with clinical thrombosis was done to investigate for hereditary prothrombotic mutations. For the neonates with thromboembolism, data were collected to reveal the underlying diagnosis, site of thrombosis, and associated risk factors. Investigations included screening for factor V 1691A and prothrombin 20210A. Seven of 9 neonates had one or more risk factors at the time of thromboembolism. Seventy percent (5/7) had underlying congenital thrombophilia (4/7 FV Leiden, 1/7 homozygote protein C deficiency). Among the healthy population, 11.9% of the neonates and 9% of the adults had FV1691A mutation, 4.8% of the neonates and 2.7% of the adults had PT 20210A mutation. Incidence of FV1691A mutation in the neonates with symptomatic thromboembolism was very high. The prevalence of both FV1691A and PT20210A mutations were remarkably higher than previously reported.*

*Keywords.* neonate, prothrombotic mutations, thromboembolism

Neonates are at a greater risk of thromboembolic complications than older children due to lower concentrations of antithrombin, heparin cofactor II, and protein C along with a reduced fibrinolytic capacity [1]. The peak incidence of thromboembolic events among children occurs in neonates and infants less than 1 year of age [2]. According to Canadian registry [3], the incidence of clinically apparent thrombosis was 2.4 per 1000 admissions to neonatal intensive care units, whereas in the German study symptomatic thrombosis was recorded to be 5.1 per 100,000 deliveries [4].

During the recent years neonatal thromboembolic events are increasingly recognized with the improvement of diagnostic tools and tertiary care neonatology. In most cases an underlying acquired or hereditary precipitating factor is present. The important acquired risk factors identified for the development of thrombosis include the presence of an indwelling catheter,

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asphyxia, sepsis, and dehydration [3, 4]. At present the role of inherited prothrombotic defects on the occurrence of thromboembolism in the neonatal period remains unclear. Evidence in the literature supporting an association between thrombotic events and specific inherited prothrombotic conditions is obtained from case reports, registry data, and small case series. The most common inherited condition causing thrombosis is factor V 1691G → A mutation, also named factor V Leiden (FVL), which leads to resistance to activated protein C [5]. Prothrombin 20210A mutation (PT 20210A), which leads to elevated prothrombin levels, can also be considered a prothrombotic condition [6].

We evaluated the prevalence of 2 common prothrombotic mutations, FVL and PT 20210A polymorphism, in 3 groups: a cohort of healthy neonates, healthy adults, and 9 neonates with symptomatic and documented thromboembolism.

## **PATIENTS AND METHODS**

A random sample of 137 healthy, term singletons born in our hospital and 368 healthy adults with no known history of familial thromboembolism constitute the cohort. We also reviewed the files of all patients who have had a thromboembolic event and were admitted to our neonatal intensive care unit (NICU) at Ankara University during the last 7 years. The total number of admissions to the neonatal intensive care unit was 2876 between the years 1994 and 2001. Data collected include gestational age, birth weight, underlying diagnosis, site of diagnosis and how the diagnosis was made and documented, and presence of risk factors for thrombosis, including presence of an indwelling catheter, sepsis, dehydration, and asphyxia. Besides investigations for thrombophilia, protein C, protein S, and antithrombin III activity, DNA was extracted and polymerase chain reactions of specific exons of FVL and PT 20210A were performed according to previously reported techniques [6, 7]. Healthy adult and neonatal cohorts were selected from the population that does not have a family history of thrombosis or stroke. Preliminary reports of the study were published previously [15–17]. Laboratory studies were performed at the Molecular Genetics and Pediatric Hematology laboratories of Ankara University. Informed parental consent was obtained for each patient and cohort case before entry in to the study. For statistical analysis, comparisons of healthy neonates and adults were made using the Pearson chi-square test.

## **RESULTS**

We evaluated 9 neonates admitted to our NICU for symptomatic thromboembolism. Two of the cases had renal vein thrombosis (RVT), 1 had purpura fulminans (PF) (Figure 1), 2 had deep venous thrombosis (DVT)



**FIGURE 1** Purpura fulminans and peripheral gangrene.

resulting in gangrenous necrosis of the upper extremities unilaterally (Figure 2), 1 had DVT of left iliac and femoral veins, and 1 had cerebral infarct due to carotid artery thrombosis (Figure 3). One neonate with congenital heart disease experienced a right atrial thrombus after catheterization. One extremely low birth weight premature infant had arterial embolism after umbilical catheterization, resulting in necrosis of right foot digits (Figure 4). Sepsis and dehydration were identified as underlying prothrombotic clinical conditions in the cases with RVT and PF, while carotid artery thrombosis was related to catheter placement. Assays for PC, PS, and AT III activity and FVL and PT 20210A mutations were conducted in 7 of the cases. Homozygous PC deficiency was diagnosed in 1 and heterozygous FVL was present in the other 4 of the cases. The prevalence of hereditary prothrombotic mutations was 5/7 among the cases with neonatal thromboembolism who could be assayed (Table 1).

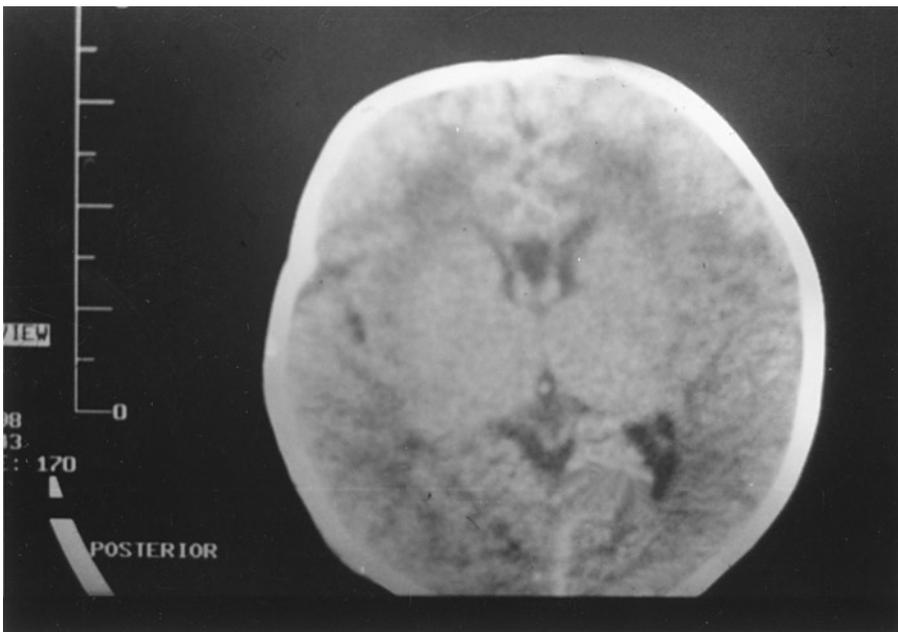
Among the healthy full-term neonates, prevalence of FVL and PT 20210G was 11.9 and 4.8% respectively. Among the healthy adults the prevalence of FVL and PT 20210A was 9 and 2.7%, respectively (Table 2).

## DISCUSSION

In the adult population, the incidence of congenital prothrombotic states and the acquired risks for thromboembolism are better described when compared with those of neonates. The congenital prothrombotic risk factors



**FIGURE 2** Gangrenous necrosis of the upper extremities unilaterally.



**FIGURE 3** Cerebral infarct due to carotid artery thrombosis.



**FIGURE 4** Arterial embolism after umbilical catheterization, necrosis of the digits.

causing thromboembolism in newborns include antithrombin, protein C, protein S, and plasminogen deficiencies and presence of PT 20210A mutation and FVL. Homozygous prothrombotic disorders usually present with severe and manifest clinical thrombosis [8]. Heterozygous congenital prothrombotic disorders in newborns also constitute a higher risk for thromboembolism, especially in the presence of confounding acquired conditions, thereby unmasking the underlying hereditary defect [9]. At present, the impact of congenital prothrombotic defects on thrombotic events occurring in the neonatal period remains inadequately defined. In our study we found a very high prevalence of (5/7) prothrombotic mutations in this selected small group of neonates with symptomatic thromboembolism. Not all patients were fully investigated so the prevalence may be even higher than what we have reported. In a study of 37 children with venous and arterial thrombosis, 5.1% of a control group, 52% with venous thrombosis, and 38% with arterial thrombosis had FVL [10]. Lawson et al. have published data from 32 children with thromboembolic episodes and 16 of their family members. The assays they performed were similar to ours and also they measured urinary homocysteine and screened for lupus anticoagulant. Forty-three percent of their cases had underlying congenital thrombophilia [11]. de Veber et al. found that 38% of the cases with cerebral thromboembolism had evidence of prothrombotic mutations [12]. In the study of Hagstrom et al. 14% of the infants and children with documented thromboembolism had FVL, and among 33 neonates

**TABLE 1** Demographic Data and Clinical Characteristics of the Cases

Case	Gestational age (weeks)	Weight at birth (g)	Risk factors	Diagnosis	Documentation	Clinical presentation	FVL vs. PT20210
1	34	2400	Sepsis dehydration	RVT	Doppler USG	Hematuria	NA
2	36	2700	Sepsis dehydration	RVT	Doppler USG	Hematuria	NA
3	38	3000		Purpura fulminans		Peripheral gangrene	+ / +, - / -; PCD
4	34	2450		Trisomy 21 DVT		Unilateral forehead gangrene	+ / -, - / -
5	39	3200		DVT	Doppler USG	Unilateral forehead gangrene	+ / -, - / -
6	40	2400	SGA catheterization	Cerebral infarct	MRI angiography	Convulsion	+ / -, - / -
7	40	3700		DVT	Doppler USG	Unilateral limb edema	+ / -, + / -
8	38	3500	Cardiac catheterization	Right atrial thrombus	Echocardiography	Congestive heart failure	- / -, - / -
9	28	750	Umbilical artery catheterization	Peripheral extremity necrosis	Doppler USG	Cold, pale extremity	- / -, - / -

*Note.* NA, not analyzed; DVT, deep venous thrombosis; RVT, renal vein thrombosis; SGA, small for gestational age; PCD, protein C deficiency; + / +, homozygote; + / -, heterozygote.

**TABLE 2** Prevalence of Prothrombotic Mutations in the Turkish Population from a Single Center

	Healthy neonates			Healthy adults			<i>p</i>
	<i>N</i> ( <i>n</i> <sup><i>a</i></sup> )	%	Allelfreq	<i>N</i> ( <i>n</i> )	%	Allelfreq	
FVL	126(15 <sup><i>b</i></sup> )	11.9	6.3	368(33)	9	4.7	.33
PT20210A	124(6)	4.8	2.4	182(5)	2.7	1.4	.33

<sup>*a*</sup>Number of cases with mutation.<sup>*b*</sup>One of the cases was homozygous.

with thromboembolism 21% were positive for FVL where this mutation was negative for 14 neonates with documented venous thrombosis [13]. Gürgey et al. also found a 50% prevalence of FVL among 12 children with thrombosis [14]. Schobess et al. found an overall frequency of 19.3 and 8.4% for FVL and PT20210A variant, respectively, among 119 children with thrombosis compared with a prevalence of 5 and 3%, respectively, in controls [15].

Strikingly high prevalence of prothrombotic mutations among our neonates (preliminary reports were published before [16–18]) led us to check the prevalence of prothrombotic mutations in healthy newborn and adult controls in our population. When compared with worldwide distribution, FV Leiden has a higher carrier rate of 9.8% in Turkish population. Prevalence of FVL mutation has been studied in various populations [19]. As initially shown in the Dutch population, this mutation has a carrier rate of 2.9%. In the study of Herrmann et al., the prevalence of FVL in populations from Poland (200), Argentina (215), Venezuela (126), Costa Rica (196), and India (150) have been reported to be 5.0, 5.1, 1.6, 2.0, and 1.3%, respectively [20].

We found the prevalence of FVL PT20210A to be relatively higher in healthy full-term neonates and healthy adults. So two distinct findings could be sought: First, prothrombotic mutations are higher in our neonates with symptomatic thromboembolism than the previously reported ones; second, healthy full-term neonates have higher prevalence of prothrombotic mutations compared to healthy adults. May we speculate that a number of neonates with prothrombotic mutations die early in life due to thromboembolic events without being diagnosed? The median age of the healthy adult cohort was 34 years. Infant mortality rate (IMR) was 153 per thousand in the 1970s in our country. This high IMR brought to our minds the question, Did some of the infants with prothrombotic mutations die of clinical conditions associated with thromboembolism before and without attaining a specific diagnosis? This may be an explanation for the difference in prevalence of prothrombotic mutations between our healthy adult and newborn groups. The high prevalence of prothrombotic mutations in a cohort of healthy neonates suggests a causal role of inherited thrombophilia in a considerable part of neonatal thromboembolism.

Data in both our own literature and world literature on inherited thrombophilia in neonatal thromboembolism are not complete. Clearly these are the areas that require multicentric well-designed investigations.

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