Coagulation factor V G1691A, factor II G20210A and methylenetetrahydrofolate reductase C677T gene mutations do not play a major role in symptomatic neonatal arterial ischaemic stroke

Genetic thrombophilia has been associated with arterial ischaemic stroke and cerebral sinus venous thrombosis in children (Kenet *et al*, 2010). However, evidence regarding neonatal arterial ischaemic stroke (NAIS) is controversial due to the retrospective nature of the studies (Miller *et al*, 2006; Suppiej *et al*, 2008; Gelfand *et al*, 2013), the recruitment of heterogeneous populations including preterm infants (Golomb *et al*, 2001; Curry *et al*, 2007; Laugesaar *et al*, 2010) or different types of neonatal stroke (i.e. presumed perinatal ischaemic stroke -PPIS) (Curry *et al*, 2007; Simchen *et al*, 2009; Laugesaar *et al*, 2010) and, more importantly, the lack of a control group (Golomb *et al*, 2001; Curry *et al*, 2007; Suppiej *et al*, 2008; Renaud *et al*, 2010).

Pregnancy itself causes a hypercoagulable state, and genetic susceptibility in the mother might also play a role in the pathogenesis of NAIS. However, genetic thrombophilia in mothers has been rarely investigated (Curry *et al*, 2007; Simchen *et al*, 2009; Renaud *et al*, 2010).

The aim of this multicentre prospective case-control study on mother-infant pairs was to determine the impact of coagulation factor V G1691A (*F5* G1691A), coagulation factor II G20210A (*F2* G20210A), and homozygous methylenetetrahydrofolate reductase [NAD(P)H] C677T (*MTHFR* C677T) gene mutations on NAIS.

From October 2006 to December 2012, 42 consecutive infants >35 weeks of gestation with the diagnosis of NAIS within the first 28 d of life, and their mothers, were recruited in three university hospitals. Eighty-five control mother-infant pairs were included. NAIS was defined according to clinical and radiographic criteria: (i) seizures, recurrent apnoea or acute neurological deficit, and (ii) magnetic resonance imaging (MRI) confirmation (sagittal and axial T1W, coronal and axial T2W and diffusion-weighted axial images) of acute focal brain infarction(s) within arterial territories corresponding to clinical manifestations. Infants with NAIS and major congenital anomalies, meningitis, sepsis, congenital infections, extracorporeal membrane oxygenation and metabolic diseases were excluded.

Blood samples from NAIS infants and their mothers were collected within 48 h of the diagnosis; controls were tested between 48 and 72 h of life, together with metabolic screening tests. The *F5* G1691A, *F2* G20210A and *MTHFR* C677T genotypes were determined by polymerase chain reaction. Regarding *MTHFR* C677T genotype, only homozygosity was considered as an abnormality.

Median age at clinical presentation was 26 h of life (interquartile range 18–43 h) and seizures were the most common presenting feature, appearing in 34/42 (81%) of the infants. Demographic data of the infants and their mothers were not significantly different between NAIS and control groups, except for birth weight and length (Table I).

Blood samples were collected at a mean age of 49·9 ± 83·8 h [95% confidence interval (CI) 24·0-74·9 h] in NAIS patients and 62·8 ± 20·9 h (95% CI 58·4–67·5) in the control infants ($P < 0{\cdot}001$). At least one mutation was found in 4 of 42 (10%) of infants with NAIS and 14 (16%) controls. Similarly, 5 (12%) mothers from the case group had one of the three mutations, compared to 13 (15%) control mothers. No differences in the three mutations tested were found between neonates with NAIS and their mothers, compared to controls (Table II).

This multicentre prospective study, carried out in infant-mother pairs, found that *F5* G1691A, *F2* 20210A and homozygous *MTHFR* C677T mutations are not more frequent in newborn infants with NAIS or their mothers.

Genetic thrombophilia plays a significant role in acute ischaemic arterial stroke in children (Kenet *et al*, 2010), however the pathogenesis in the perinatal period is probably different. Our data support the findings of previous neonatal studies on *F2* G20210A and homozygote *MTHFR* C677T, which have not found association of these mutations with NAIS (Miller *et al*, 2006; Gelfand *et al*, 2013; Laugesaar *et al*, 2010; Günther *et al*, 2000). On the other hand, *F5* G1691A mutation has been associated with NAIS in some controlled studies (Günther *et al*, 2000; Simchen *et al*, 2009), but not in others (Miller *et al*, 2006; Laugesaar *et al*, 2010; Gelfand *et al*, 2013). In our study, *F5* G1691A mutation was found in only 5% of the infants with NAIS and their mothers, compared to 2% in the control group without significant differences. Large-scale case-control studies are required to better define the role of *F5*.

Genetic thrombophilia in mothers of newborn infants with arterial ischaemic stroke has been previously evaluated in three studies with disparate results (Curry *et al*, 2007; Simchen *et al*,

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Table I. Demographic characteristics of 42 infants with NAIS and their mothers compared with 85 controls

	All (N = 127)	Cases (N = 42)	Controls (N = 85)	P
Maternal findings				
Maternal age, years	33 ± 5 (20;44)	32 ± 5 (20;43)	33 ± 4 (21;44)	0·936
BMI before pregnancy*	24 ± 4 (18;40)	23 ± 4 (18;40)	24 ± 4 (18;39)	0·264
Maternal ethnicity				
Caucasian	114 (89)	36 (88)	78 (94)	0·220
Arabian	8 (6)	4 (10)	4 (5)	
Asiatic	1 (1)	1 (2)	0	
African	2 (2)	1 (2)	1 (1)	
Primiparity	76 (60)	24 (57)	52 (61)	0·663
Infant findings				
Gestational age, weeks	39·5 ± 1·2 (35; 41)	40 ± 1·3 (35; 41)	39 ± 1·2 (37; 41)	0·233
Male to female ratio	61:66	22:20	39:46	0·490
Birth weight, kg	3·28 ± 0·414 (2·25; 4·3)	3·164 ± 0·391 (2·25; 4·11)	3·337 ± 0·415 (2·4; 4·3)	0·026
Birth length, cm	50 ± 1·7 (45; 54)	49·5 ± 1·7 (45; 53)	50 ± 1·5 (47; 54)	0·001
Birth head circumference, cm	34·4 ± 1·2 (31; 37)	34 ± 1·1 (32; 37)	34·5 ± 1·1 (31; 37)	0·504

Quantitative variables are expressed in median ± standard deviation (minimum; maximum) or in n (%) when necessary.
NAIS, neonatal arterial ischemic stroke; BMI, body mass index.
*Data from BMI were obtained from 30 cases and 77 controls

Table II. Thrombophilia results for the three mutations tested (*F5* G1691A, *F2* G20210A and *MTHFR* C677T) in infants with neonatal arterial ischemic stroke (NAIS) and their mothers, compared to controls

	Cases n/N (%)	Controls n/N (%)	P
F5 G1691A			
Newborn	2/42 (5)	2/85 (2)	0·599
Mother	2/42 (5)	1/85 (1)	0·254
Newborn and mother	1/42 (4)	0/85	–
F2 G20210A			
Newborn	1/42 (2)	1/85 (1)	1·000
Mother	2/41 (5)	3/85 (4)	0·660
Newborn and mother	1/41 (4)	0/85	–
MTHFR C677T			
Newborn	1/35 (3)	11/81 (14)	0·104
Mother	1/34 (3)	9/82 (11)	0·277
Newborn and mother	0/34	2/81 (3)	–

There were no cases of homozygous *F5* G1691A or *F2* G20210A mutations.
There were no cases of infants or mothers with two or three mutations. There were six pairs in which both mother and child exhibited thrombophilia, two in the NAIS group (5%) and four (5%) in the control group. In four cases, both the infant and the mother presented the same mutation and two infant-mother pairs had combined mutations of different genetic factors: one had combined *F5* G1691A (infant) and *F2* G20210A (mother) mutation, and another had combined *MTHFR* C677T homozygote (infant) and *F5* G1691A (mother) mutation.

2009; Renaud *et al*, 2010). One study each found an association for *MTHFR* C677T (Curry *et al*, 2007) and *F5* G1691A mutation (Simchen *et al*, 2009) in mothers of infants with NAIS. We did not find any association (Renaud *et al*, 2010), and in contrast to those studies, mothers in our series were not analysed less frequently than children.

Our study has the limitation of a relatively small size cohort, though very few case-controlled studies reported higher number of infants with NAIS (Günther *et al*, 2000; Laugesaar *et al*, 2010). In addition, it has several strong points that may partly explain the differences we found compared to other series. It is a prospective study with a control group and a homogeneous population with a common ethnic background, comprising exclusively symptomatic neonates whereas other studies included infants without clinical symptoms (i.e. with PPIS) (Curry *et al*, 2007; Simchen *et al*, 2009; Laugesaar *et al*, 2010). Of note, the influence of thrombophilia may be more relevant in the pathogenesis of PPIS than in the NAIS group (Golomb *et al*, 2001; Suppiej *et al*, 2008). In addition, all of the infants in our study were diagnosed with MRI, while others have used cranial ultrasound or computed tomography (Günther *et al*, 2000; Simchen *et al*, 2009; Laugesaar *et al*, 2010; Renaud *et al*, 2010), that have more limitations to detect small and peripheral ischaemic lesions.

In conclusion, the routine screening of patients with NAIS for *F5* G1691A, *F2* G20210A and *MTHFR* C677T gene mutations might not be justified, and additional prothrombotic mechanisms should be considered.

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Authorship and disclosures

JA, GA, AMA and AGA contributed to data acquisition and interpretation; JA analysed the data; JA and AGA wrote the paper and GA and AMA revised it critically. AGA designed the research study. The authors have no financial conflicts of interest.

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