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Inherited and Acquired Risk Factors and Their Combined Effects in Pediatric Stroke

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The aim of this study was to identify hereditary and acquired risk-factors as they are related to the occurrence of stroke in children. We identified 21 children with stroke. A search of the Factor V Leiden mutation, the Factor II G20210A variant, and the thermolabile variant of methylenetetrahydrofolate reductase was performed in patients and in a control group (n = 115).

We identified risk factors of acquired and/or hereditary nature for stroke in 19 of 21 children. Eleven children had three or more risk factors, seven had two risk factors, and one child had only one risk factor. We found three carriers (14.3%) of the Factor V Leiden mutation, two carriers (9.5%) of the Factor II G20210A variant, eleven (52.4%) thermolabile variant of methylenetetrahydrofolate reductase heterozygote carriers, and one (4.8%) homozygotes for this variant.

Frequencies of the Factor V Leiden mutation and the Factor II variant were higher in patients than in controls, suggesting that these variants are associated with an increased risk of stroke in childhood. Homozygosity for the thermolabile variant of methylenetetrahydrofolate reductase was equally frequent amongst patients and controls.

Our study confirms that stroke in children is commonly associated with a combination of multiple risk factors, both genetic and acquired, and that the Factor V Leiden mutation and the Factor II G20210A variant are predisposing factors for this situation.

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Introduction

Pediatric stroke is more common than previously thought [1]. Its overall incidence has been estimated to be 2.5 cases in 100,000 per year [2], with 44% to 61% of ischemic origin [2-4]. Awareness of etiologic factors is essential because recurrence is significant and disabilities are frequent, endangering quality of life [1,5,6]. Several conditions have been associated with stroke, including cardiac disease, vascular abnormalities, endothelial damage, infectious diseases, collagen tissue disease, and some inborn errors of metabolism and inherited or acquired coagulation abnormalities that predispose to thrombotic complications. Reported prothrombotic abnormalities include anticardiolipin antibody, lupus anticoagulant, deficiencies of protein C, protein S, antithrombin, and plasminogen [1,5-11]. New hypercoagulable states are being identified as risk factors for ischemic stroke in childhood: the presence of abnormal activated protein C resistance (or Factor V Leiden), Factor II G20219A variant, and thermolabile variant of methylenetetrahydrofolate reductase (MTHFR C677T) [8-13]. The knowledge of such abnormalities is important to define more rational prophylactic and therapeutic strategies.

The aim of this study was to identify thrombophilic conditions that might be related to the development of stroke in children.

Patients and Methods

This retrospective study included 21 children with ischemic stroke observed in the last 12 years (1987-1999) at Hospital de Crianças Maria Pia. Neonatal stroke, as well as patients presenting with transient symptoms, were excluded from this series. The diagnosis of cerebral ischemic stroke was confirmed in all patients by computerized tomography (CT) and/or magnetic resonance imaging (MRI) scans. Addition-

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ally, magnetic resonance angiography (MRA) was performed in three patients, standard angiography in four, and one child had MRA and standard angiography.

Data collected included age at diagnosis, sex, previous medical history, family history, signs and symptoms presented, radiologic findings, treatment, and outcome.

Baseline investigations performed at the time of diagnosis included complete blood count, blood sedimentation rate and/or C-reactive protein, liver-function tests, creatinine, blood urea nitrogen, electrolytes, total cholesterol, triglycerides, lipoprotein (a) [Lp(a)], VDRL, chest radiography, electrocardiogram, and echocardiogram. Serologic tests, immunologic profile, muscle biopsy, skin biopsy for fibroblast characterization, and evaluation of cerebrospinal fluid pyruvate and lactic acid levels were also performed when indicated to establish a specific etiology.

The thrombophilia investigation included prothrombin, activated thromboplastin, thrombin and reptilase times, plasma fibrinogen level, activity of protein C, protein S, antithrombin III and plasminogen, normalized activated protein C sensitivity ratio (n-APC-SR), total homocysteine plasma level, lupus anticoagulant, anticardiolipin, and anti-β2 glycoprotein1 antibodies.

DNA analysis, for detection of Factor V Leiden mutation, Factor II G20219A variant, and thermolabile variant of methylenetetrahydrofolate reductase, was performed both in patients and in a control population (n=115) of Portuguese origin, matched by sex and region of origin to the patient sample. DNA from controls was obtained from anonymous Guthrie cards (newborn screening of phenylketonuria) by the Chelex method. DNA from patients was extracted from peripheral blood by standard methods. Identification of the Factor V Leiden mutation (G1691A) [5] and Factor II G20210A variant [6] was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with MnlI and HindIII, respectively. The MTHFR variant was assessed by PCR-RFLP with HinfI in the control population, and by sequencing (using the primer 5′;TGGGCCCCTCACCTGGATG3′;) of the purified PCR fragments in patients.

Fisher's exact test was used to compare the frequency of risk genotypes in patients and in controls. Odds ratios, with a 95% confidence interval, were estimated as an approximation of relative risk, given the low frequency of risk factors. A *P*-value less than 0.05 was considered to be statistically significant.

Results

Clinical Findings

The main clinical findings of our patients are summarized in Table 1: 67 percent were female. The age range was two months to 13 years with a mean of 5.3 years. Ten patients had a prior medical history with conditions predisposing to stroke. Considering family history, only one child had a father with myocardial infarction by the age of 45.

Treatment and Outcome

At the acute stage, treatment was only symptomatic. Heparin therapy was used in three patients: cases 6 and 7 had a corticosteroid dependent nephrotic syndrome, and case 8 had a cyanotic cardiac disease. Antiplatelet prophylaxis was instituted in eight patients (seven with aspirin and one with indobufene).

Total recovery was achieved by 11 patients (52.4%). Persistent motor deficit (hemiparesis in seven patients and quadriparesis in two), symptomatic epilepsy (five patients), and cognitive impairment (seven patients) were the main sequelae observed. Five of the eight patients with seizures at the onset of stroke later developed epilepsy. Disabilities were as frequent in our patients (47.6%) as reported by others [7]. Recurrence of stroke occurred in five patients (23.8%), a frequency similar to previous reports [5,11,14].

Risk Factors

Known risk factors for stroke were identified in 19 children (Table 1). Risk factors of a hereditary nature were identified in 10 patients, whereas acquired risk factors were found in 18 patients. Eleven patients (52.4%) had three or more risk factors, seven (33.3%) had two, and one (4.8%) had only one. In two patients, no risk factors were identified.

All recurrences (cases 3, 8, 13, 16 and 18) occurred in children with identified risk factors (Table 1); these cases however had a longer follow-up than those without recurrences (10.5 vs 7 years).

Genotype distributions for the Factor V Leiden mutation, Factor II, and MTHFR variants are given in Table 2. Three of the 21 patients (14.3%) were heterozygous for Factor V Leiden. Two patients (9.5%) carried the Factor II G20210A variant. The T/T genotype at position 677 of MTHFR was present in one case (4.8%). When compared with controls, the difference in allele frequencies at the Factor V and Factor II loci was not statistically significant ($P = 0.074$ and $P = 0.064$, respectively), but exhibited a trend towards increased frequency of these variants amongst patients. Odds ratios were 4.63 (95% CI: 0.95-22.40) and 11.79 (95% CI: 1.02-136.52), suggesting that carriers of these variants have approximately 5-fold and twelve-fold higher risks of developing stroke than non-carriers. Homozygosity for the MTHFR variant is equally frequent amongst patients and controls.

Discussion

A significant number of patients (90%) in our series had one or more predisposing conditions for stroke. MRA and/or standard angiography was performed in just eight patients, and these studies would have identified additional risk factors. Only 10% did not have any recognizable risk factor, which is less than the 20-33% referred in literature [1,2,5,11,12,15]. This difference probably reflects different depths of investigation. The most frequent risk factor was the presence of antiphospholipid antibodies (62%), as reported in other studies [5,9,12,15]. Increased Lp(a) was found in 29% of the children, which is also in agreement with recent reports that consider it as an important risk factor for spontaneous ischemic stroke in childhood [12,13].

We have provided evidence that both Factor V Leiden mutation and Factor II G20210A variant have influence as risk factors for stroke in our pediatric population (relative risks of 4.63 and 11.79, respectively).

Table 1. Clinical characteristics of the patients - Summary

Patient	Sex	Age at stroke	Presentation	Infarct localization or vascular involvement	Risk factors	
					Acquired	Inherited
1	M	5yr 5mo	Cerebellar syndrome (+ L), R hemiplegia	Bilateral cerebellar hemispheres (+ L), pontomesencephalic transition	Dissection vertebral artery, cervical trauma, lupus anticoagulant	Lp(a) > 30mg/dl
2	M	6yr	Cerebellar syndrome (+ R), R hemiplegia, cephalalgia	L pons, R median cerebellar peduncle, L paramedian occipital	Dissection basilar artery, anti b2-glycoprotein I Ab	
3	F	17mo	L amaurosis, L ptosis, L hemiplegia			
		5yr 2mo	L hemiplegia			
		5yr 5mo	R hemiplegia, aphasia			
		6yr	Bilateral amaurosis	Bilateral cerebral hemispheres, retina	Sneddon syndrome, anti-cardiolipin Ab	
4	F	12yr	Seizures	Bilateral parietal + occipital	Systemic lupus erythematosus, anti-b2-glycoprotein I Ab	Lp(a) > 30mg/dl
5	F	12yr 10mo	Focal seizures	Bilateral frontal + occipital	Systemic lupus erythematosus, anti-b2-glycoprotein 1 Ab, lupus anticoagulant, hyperhomocystinemia, hyperlipidemia	
6	F	6yr 7mo	Cephalalgia, vomiting, convergent strabismus	LSS thrombosis	Nephrotic syndrome, hyperlipidemia, anticardiolipin Ab	
7	M	5yr	Cephalalgia, vomiting	LSS thrombosis, L frontal + parietal	Nephrotic syndrome, hyperlipidemia	FV Leiden
8	F	1yr 6mo	R hemiplegia, aphasia			
		1yr 10mo	L hemiplegia	R temporoparietal	Cyanotic cardiac disease, heart surgery	
9	M	4yr	L hemiplegia	R pons	Cyanotic cardiac disease, anticardiolipin Ab	Lp(a) > 30mg/dl
10	F	5yr	L hemiplegia	Basal ganglia	Heart surgery, endocarditis, hyperlipidemia, lupus anticoagulant	
11	F	1yr 11mo	L hemiplegia	Basal ganglia	Myocarditis, airways infection	
12	F	4yr 6mo	Cephalalgia, amaurosis	Vertebrobasilar	Chronic renal insufficency hypertension, transitory systemic hypotension, hyperlipidemia, lupus anticoagulant	
13	F	7yr	R hemiplegia,			Fanconi's anemia,
		7yr 1mo	L hemiplegia	Bilateral cerebral hemispheres	Hyperlipidemia, hypertension	Moyamoya syndrome
14	M	6yr	R hemiplegia	L pons	Obesity	FV Leiden, Lp(a) > 30mg/dl
15	M	1.5 mo	Status epilepticus, coma	Bilateral cerebral hemispheres	Meningitis, anticardiolipin Ab	FV Leiden, Protein S deficiency
16	M	13yr	Seizures, coma			
		13 yr + 3 mo	Dystonia, tetraparesis	R cerebral hemisphere	Meningitis, anticardiolipin Ab	Lp(a) > 30 mg/dl
17	F	2mo	Seizures	Bilateral cerebral hemispheres		FII variant Lp(a) > 30mg/dl
18	F	13mo	L hemiplegia, seizures	R cerebral hemisphere		
		18mo	R hemiplegia, seizures	L parieto-occipital	Airways infection, anticardiolipin Ab	FII variant
19	F	18mo	R hemiplegia, seizures	Bilateral cerebral hemispheres	Anticardiolipin Ab	
20	F	4yr	Seizures	R frontoparietal		
21	F	4yr	R hemiplegia	Basal ganglia		

Abbreviations:
+ = Lateral predominance
Ab = Antibody
L = Left
LSS = Longitudinal superior sinus
R = Right

Table 2. Genotype frequency of patients and controls. Shaded boxes indicate suspected risk genotypes; Data was grouped for analysis according to presence or absence of risk genotype

Genotype	Observed Frequency (%) Patients (n = 21)	Control group (n = 115)	$P^{\ddagger}$	Odds ratio (95%CI)
FV Leiden (G1691A)				
G/G	18 (85.7)	111 (96.5)	0.074	4.63 (0.95-22.40)
G/A	3 (14.3)	4 (3.5)		
A/A	0 (0)	0 (0)		
FII variant* (G20210A)				
G/G	19 (90.5)	112 (99.1)	0.064	11.79 (1.02-136.52)
G/A	2 (9.5)	1 (0.9)		
A/A	0 (0)	0 (0)		
MTHFR† (C677T)				
C/C	9 (42.8)	55 (48.2)	0.298	0.37 (0.05-2.98)
C/T	11 (52.4)	46 (40.4)		
T/T	1 (4.8)	13 (11.4)		

*,† — Two, one controls did not amplify, respectively
‡ — 2×2 Fisher's exact test

More importantly, however, stroke emerges as a condition associated with a combination of multiple risk factors, both inherited and acquired.

It is noteworthy that stroke recurrence occurred only in patients with recognizable risk factors, as reported by other series of patients [5,11,12]. This finding highlights the importance of their identification in the definition of prognosis and in the management decisions (treatment and secondary prophylactic measures).

The treatment of stroke in children has been primarily directed toward stabilizing systemic factors and managing underlying causes. No randomized, controlled treatment trials have been completed in children who suffered stroke. Many of the treatment approaches have been adapted from studies in adults, which include antithrombotic therapies (heparin, low molecular weight heparins, aspirin, warfarin, and thrombolytic agents), neuroprotective agents, and immunosuppressive therapy.

Accumulating experience with antithrombotic and anticoagulant treatment in children suggests that these agents can be used safely, although their efficacy and proper dose still need to be established. Low molecular weight heparin is increasingly used as the first choice for acute anticoagulant therapy in children with arterial ischemic stroke (AIS); its indications include arterial dissection, coagulations disorders, embolism from the heart (usually complex congenital heart disease), and children with progressive or additional neurologic deficits not caused by cerebral hemorrhage during the initial evaluation of a new cerebral infarction. Aspirin is frequently used as the treatment of choice for secondary prevention of AIS in situations of increased risk of recurrence such as cerebral arterial stenosis. Currently, most older children with AIS are placed on aspirin even when no cause is identified because of the risk of recurrence and minimal side effects. Major uses of warfarin treatment in children include congenital or acquired heart disease, severe hypercoagulable states, arterial dissection, and recurrent AIS or transient ischemic attacks while on aspirin [11,15]. In our study, none of the patients treated with heparin and/or aspirin had recurrence, but the restricted number of the cases limits the conclusion. Large controlled clinical trials are required to establish the role of antithrombotic and other therapies.

In conclusion, our study confirms that stroke in children is associated with a combination of predisposing conditions, genetic and acquired, often with the presence of multiple risk factors.

The most important risk factors identified were antiphospholipid antibodies and increased lipoprotein (a). The Factor V Leiden mutation and Factor II G20210A variant seem to be relevant predisposing factors for stroke in children of our study. Further analysis of these risk factors in larger series of pediatric patients would contribute to validate these findings. The fact that recurrence occurred only in children with identified risk factors suggests that identification of these factors should be considered for the management of such cases.

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