Thrombophilia: A Risk Factor for Cerebral Palsy?

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Abstract

Background: The cause of cerebral palsy remains unknown in most cases. Factor V Leiden mutation, a common cause of hereditary thrombophilia, has been associated with CP.

Objectives: To analyze the prevalence of factor V Leiden (*G1691A*), prothrombin (*G20210A*), and methylenetetrahydrofolate reductase (*C677T*) mutations in children with CP.

Methods: Sixty-one Jewish and Arab children with CP were studied for the presence of the three gene mutations associated with thrombophilia.

Results: We found that 41% of the children with CP and 33% of the controls carry one or more of the studied mutations ($P = 0.348$). The prevalence of the factor V mutation was 27.9% in CP and 16.4% in controls ($P = 0.127$). The frequency of the other two genetic factors was even less significant. The FVL mutation was found in 35% of the Arab CP patients (15/42) and in 22% of the controls from the same population (9/40) ($P = 0.067$).

Conclusions: Each of the genetic factors studied was shown to be related to CP. Despite the high frequency of FVL among the studied patients, we were unable to prove a significant correlation between FVL and CP, mainly because this factor is frequent in the Arab control group. In this population a trend toward significance can be seen ($P = 0.067$). Larger studies are needed to validate the significance of these results.

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Cerebral palsy, one of the most common neurologic disorders of childhood, remains a major cause of chronic disability. CP is defined as a disorder of the central nervous system manifested by aberrant control of movement or posture caused by a non-progressive brain insult in early life.

In the past few years, enormous efforts have been invested in the attempt to identify factors associated with the risk of developing CP and several etiologies were proposed. Asphyxia, genetic and neuronal disorders, infection *in utero*, amnionitis, maternal autoimmune disease, metabolic disease and coagulation disorders were all suggested as risk factors for CP [1–5]. Vascular lesions, most often mediated by thrombi, were recently associated with hemiplegic CP [6–10].

Thrombophilia is an acquired or hereditary hypercoagulable state that is related to a high incidence of thrombosis. Among the genetic causes of thrombophilia we found deficiency of pro-

CP = cerebral palsy
FVL = factor V Leiden

tein C, protein S, and antithrombin III; high levels of factors VIII and XI; mutation in the prothrombin gene (*G20210A*); resistance to activated protein C caused by mutation in the coagulation factor V, the factor V Leiden mutation (*G1691A*); and hyperhomocystinemia caused by point mutation in the gene encoding 5,10-methylenetetrahydrofolate reductase (*C677T*). Acquired risk factors for thrombosis include immobilization, major surgery, pregnancy, oral contraceptives, central venous lines and antiphospholipid syndrome associated with lupus anticoagulant or anticardiolipin antibodies [11–15].

We performed a case-control study in children with CP to determine whether the gene mutations related to thrombophilia are more prevalent in children with CP. The demonstration of such an association (between CP and thrombophilia) carries huge potential for the diagnosis and prevention of CP.

Patients and Methods

Children aged 6 months to 18 years with spastic CP were recruited from a population treated at HaEmek Medical Center in Afula, Israel. The hospital is a district teaching hospital serving a population of 500,000, of whom 35% are younger than 18 years. In this young population, nearly half are of Arab ethnicity.

Cerebral palsy was defined as a chronic static disability of the central nervous system characterized by aberrant control of movement or posture and appearing early in life. CP was determined by standardized examination and record review. Patients were recruited during the years 2000 to 2004 from children with CP admitted to the Unit of Child Development and the Pediatric Orthopedic Surgery CP Clinic. Children with disability arising from infection, trauma, or adverse events after the neonatal period were excluded. We approached 68 families; 7 refused to participate in the study. The final sample consisted of 61 children with spastic CP for whom clinical data and blood samples were available. Informed consent was obtained from the parents or guardians during the interview.

Data were obtained from the medical files and by interviewing the child or parents. The data included demographic characteristics, consanguinity, pregnancy, delivery, birth weight and perinatal events. Family history of thrombophilia and other neurologic disorders were also recorded. All patients were clinically examined and neurologic findings recorded.

Control DNA samples were obtained from 62 children aged 6 months to 18 years who were evaluated in the outpatient clinic for either short stature or familial Mediterranean fever. None had a neurologic disorder or clinical symptoms related to the coagulation system. We obtained consent to use the DNA samples for research purposes. Controls were not interviewed, thus individual identifiers other than age, ethnic origin and medical diagnosis were not available. The controls were matched by ethnic origin and age. The study was approved by the hospital Helsinki Committee.

Laboratory tests

The patients were screened for inherited defects in coagulation. We determined protein C, protein S and antithrombin III activity in the patient group only. Genetic analysis of all the children was performed to determine the presence of mutations in FVL (*G1691A*), prothrombin gene (*G20210A*), and MTHFR (*C677T*). Genomic DNA was extracted from peripheral blood according to Miller and Polskey [16]. The presence or absence of FVL (*G1691A*), MTHFR (*C677T*), and prothrombin gene (*G20210A*) mutations was determined by using the GamidaGen-pronto Thrombo risk kit (Petah Tikva, Israel). For confirmation of the genotypes, an allele-specific restriction enzyme analysis was performed. The results of the allele-specific restriction enzyme analysis were in 100% concordance with the results of genotype analysis as determined by the GamidaGen-pronto kit.

Statistical analysis

The SPSS statistical package was used for all statistical analysis. The association between the categorical variables was assessed by Pearson Chi-Square or by Fisher's exact test for small groups. Differences at the $P \leq 0.05$ level were considered statistically significant.

Results

Group characteristics

The study group comprised 61 CP patients and 62 controls. Patients and controls were similar with regard to gender distribution, gestational age and ethnic origin. The median age was 9 years (range 0.6–18 years) in the CP group and 7 years (range 0.6–18) in the control group. In both groups, the majority was Arab – 69% (42/61) in the patient group and 66% (41/62) in the control group; the other patients and controls were Jewish.

In the patient group we recorded consanguinity in 40% (25/61); all were Arabs (25/42). Twelve (19%) had a family history of motor neurologic disorder, of whom 3 had a sibling with CP. Those three siblings were not evaluated due to lack of consent or unavailability.

All patients had spastic CP: 28 (46%) were hemiplegics, 14 (23%) quadriplegic, 18 (29%) diplegic and one monoplegic. Seventeen patients (28%) had a first-degree relative with a history of thrombotic event. Only one patient had a thrombotic event beyond the neonatal period, an adolescent who developed

deep vein thrombosis after orthopedic surgery. Another subject sustained a prenatal and postnatal brain infarct and renal and inferior vena cava vein thrombosis within the first 48 hours after birth. Both children had a positive family history of thrombophilia and neither had brain infarct diagnosed after infancy.

The protein C, protein S and antithrombin III results of the patients were in the normal range or at non-significant slightly low levels (Data not shown).

Prevalence of inherited risk factors for thrombophilia [Table 1]

The prevalence of the FVL mutation was higher among CP patients than among the controls but the difference was not significant ($P = 0.127$). The case and control distribution for the prothrombin and MTHFR (homozygous) mutations was similar. Overall, 25 of the 61 children with CP (41%) and 20 of the 62 controls (33%) had one or more of the three mutations ($P = 0.348$). Thirteen of the CP patients had a positive family history of thromboembolic disease; 6 of them have thrombophilia. In the two patients who had thromboembolic events after birth, the genetic analysis was positive and consistent with thrombophilia. A positive family history of neurologic disorder was found in 12 subjects, of whom 5 have thrombophilia (41%). The genetic profile for two of the three children who have a sibling with CP was positive for thrombophilia.

Prematurity and abnormal intrapartum events

Of 21 patients who were born prematurely (36%), 11 had a positive genetic profile for thrombophilia, almost twice as many as term patients (52% vs. 35%, $P = 0.2$). The genetic mutations found were FVL (7/21, 33%), homozygous for MTHFR mutation (3/21, 14%), and prothrombin mutation (1/21, 4%). Five of the premature children had undergone umbilical catheterization, and three of them were positive for genetic mutations.

Abnormal intrapartum events were documented in 24 children (41%). The prevalence of thrombophilia was not significantly different among patients with or without abnormal intrapartum events ($P = 0.296$).

Table 1. Prevalence of mutations in the genes for factor V Leiden *(G1691A)*, prothrombin *(G20210A)*, and methylenetetrahydrofolate reductase *(C677T)* in children with CP and controls

Type of mutation	CP group (n=61) No. (%)	Control group (n=62) No. (%)	P
FVL (*G1691A*)			
Heterozygous	16 (26%)	7 (11.5%)	0.127
Homozygous	1	3	
MTHFR genotype (*C677T*)*	9 (15%)	8 (13.1%)	0.794
Prothrombin genotype (*G20210A*)	5 (8.5%)	5 (8.2%)	0.956
Total**	25 (41%)	20 (32.8%)	0.348

* Heterozygous for the *MTHFR* mutation and therefore excluded from the analysis.

** Includes all positive genetic analysis. Several children presented with a combination of mutations: 3 CP patients and 3 in the control group had FVL mutation and were homozygous for MTHFR, 1 CP patient had a mutation in factor II and was homozygous for MTHFR, and 2 in the CP group were heterozygous for all three mutations.

MTHFR = methylenetetrahydrofolate reductase

Brain imaging and CP type

Brain imaging was performed in 47 patients (77%): 36 had computed tomography, 5 had both CT and magnetic resonance imaging, 5 had MRI (22%), and 1 had brain ultrasound. Results were available for 46 cases, and 29 (66%) were interpreted as abnormal. Twenty-five (86%) had a lesion that could be the result of a vascular insult; 14/18 had an infarct. We found no correlation between hemiplegic CP, abnormal brain imaging and thrombophilia.

Ethnic origin

The FVL mutation was detected in 35% of the Arab CP patients (15/42), and in 22% of the controls from the same population (9/40) ($P = 0.067$). The FVL mutation was also found in two of the Jewish patients but in none of the controls of the same ethnic origin (not significant). The frequency of FVL was significantly higher in Arab patients and controls compared to Jewish patients and controls ($P = 0.001$). There was no difference between Jewish and Arab patients and controls for the other mutations.

Discussion

Thrombophilia is considered a cause of cerebrovascular events in children. Recent reports have linked thrombophilia with CP [6–10]. Three case reports [6–8] demonstrated the relationship of FVL mutation and hemiplegic CP. Lynch and colleagues [9] summarized the literature on children with cerebrovascular disorder and the FVL mutation. They also described eight children with CP who had elevated levels of the translational product of the FVL mutation. The first population study investigated thrombophilia in children with hemiplegic CP only [10]. In that study Smith et al. evaluated 27 children and their findings suggested that thrombophilia is not a cause of hemiplegic CP. In the present study we investigated all types of spastic CP and compared the frequency of the genetic mutations with a control group.

The FVL mutation is the most common hereditary cause of thrombophilia, accounting for 60–70% of familial thrombophilia [17], and its prevalence in Caucasians ranges from 3% to 7%. Data regarding the ethnic prevalence of hereditary thrombophilia in Israel are lacking. Unpublished results of an ongoing study performed in our center on healthy Arab women showed that nearly 20% were heterozygous for the FVL mutation. Another study performed in Israel by Brenner et al. [18] found that the frequency of the FVL mutation is about 10%, but they did not specify the various ethnic groups. In our study, the rate of the FVL mutation in the control group was 16.4%, but the proportion of Arab controls was high and among this group the frequency of FVL was significantly high. FVL mutation was positive in 35% and in 22% of the Arab children in the patient and control groups respectively. This mutation was not found in the Jewish control group.

A high prevalence of congenital and Mendelian diseases is representative of our Arab population, where consanguinity in more than 50% of the couples is common. Zlotogora and asso-

ciates [19] showed that the high incidence of congenital diseases in Israel's non-Jewish population parallels the elevated rate of consanguinity in that population. A survey performed among Israeli Arabs found a 42% rate of consanguinity among Moslems [20]; therefore our findings are representative of the population studied. A survey performed in the United Kingdom found that CP was more prevalent among Asians, where consanguinity was more common (51.7%), compared to a non-Asian population (5.48–6.42 vs. 3.18 per 1000 respectively) [21]. There is no similar survey in the Israeli population. We can only assume that in a community where consanguinity is common, CP tends to be more related to genetic diseases, such as thrombophilia.

Nearly one-third of the CP patients in our study were born prematurely. In 52% of the premature CP patients the genetic analysis was positive for thrombophilia (vs. 35% of non-premature CP patients, $P = 0.2$). The perinatal and neonatal periods predispose the fetus as well as the infant to thrombotic events. In the fetus the thrombotic system is tilted slightly in favor of thrombosis; fetuses have a high hematocrit and relatively slow blood flow. They also have a patent foramen ovale, which allows venous emboli to enter the arterial system, and more than 60% of fetal cardiac output goes to the brain, which would predispose it to cerebral infarction [6]. Hence, immaturity of the anticoagulant system, venous catheterization, polycythemia, and other risk factors for thrombosis in a genetically predisposed baby all contribute to thrombotic events. Prematurity itself is a risk factor for CP, and this tendency for thrombosis could present an additional risk.

Contrary to our expectations, brain imaging did not demonstrate a relationship linking infarct, thrombophilia and CP. The superiority of MRI over CT in examining CP children has been well documented [22]. Many of the studies on MRI in CP patients demonstrated significant findings in most patients and were helpful in depicting an etiology [22–25]. The lack of correlation between the imaging findings and thrombophilia in our study could be related to the fact that MRI was not performed in most of the cases.

Our study had certain important limitations. The sample size and the possibility of other genetic causes in a consanguineous population preclude our ability to determine whether thrombophilia causes CP. The correlation between FVL and CP should be studied in a larger group of patients of Arab origin. Furthermore, we obtained data years after the presumed thrombotic event occurred, while in the critical period around birth there could have been additional prothrombotic risk factors. In addition, we randomly chose children with spastic CP, yet it is reasonable that CP of vascular etiology would be present in children who display focal neurologic disability. Therefore, specific cerebral palsy subtypes should be studied as outcomes, because there is evidence that the different subtypes may be etiologically distinct [2,6,7,23].

In presenting our preliminary results we suggest that FVL mutation be considered as a risk factor for CP, in addition to other risk factors that are likely involved in producing brain injury. We were not able to prove a significant correlation be-

tween FVL and CP, mainly because this factor was a frequent finding in the Arab control group and probably represents the true frequency in this population. Our study proposes that thrombophilia leads to brain vascular insult and could be a mechanism of CP development during the neonatal period. Thrombophilic risk factors and more specifically the FVL mutation should be considered in the evaluation of selected patients with CP. Further understanding of the risk factors involved in the development of CP may help in creating treatment modalities, such as anticoagulant treatment for mothers, in order to prevent this disability.

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> Each man takes care that his neighbor shall not cheat him. But the day comes when he begins to care that he does not cheat his neighbor. Then all goes well – he has changed his market-cart to a chariot of the sun
>
> Waldo Emerson (1802-82), American writer and philosopher

> Finish every day and be done with it. You have done what you could. Some blunders and absurdities crept in. Forget them as soon as you can. Tomorrow is a new day. You shall begin it serenely and with too high a spirit to be encumbered with your own nonsense.
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> Ralph Waldo Emerson (1803-82), American writer and philosopher