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Associations between inherited thrombophilias, gestational age, and cerebral palsy

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KEY WORDS
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Objective: This study was undertaken to investigate associations between inherited thrombophilic polymorphisms and cerebral palsy (CP) in a large case-control study.

Study design: This is a population-based case-control study. Genomic DNA from newborn screening cards of 443 white CP cases and 883 white controls was tested for factor V Leiden (FVL, G1691A), prothrombin gene mutation (PGM, G20210A), and methylenetetrahydrofolate reductase (MTHFR) C677T and MTHFR A1298C.

Results: MTHFR C677T was associated with an increased risk of developing any CP (32-36 weeks' gestation, homozygous odds ratio [OR] 2.55, 95% CI 1.12-5.74; heterozygous OR 1.91, 95% CI 1.01-3.66). MTHFR C677T was also associated with diplegia at both less than 32 weeks' gestation (homozygous OR 2.76, 95% CI 1.21-6.12) and all gestations (heterozygous OR 1.58 95%, CI 1.02-2.45). For children less than 32 weeks, FVL homozygosity may be associated with an increase in the risk of developing quadriplegia (OR 9.12, 95% CI 0.86-53.71). MTHFR A1298C (heterozygous) was associated with a reduced risk of diplegia developing at 32 to 36 weeks' gestation (OR 0.16, 95% CI 0.02-0.70). There were no associations between any type of CP and thrombophilia for children born 37 weeks or greater. Heterozygous PGM and homozygous MTHFR C677T combined were associated with quadriplegia at all gestational ages (OR 5.33, 95% CI 1.06-23.25).

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Conclusion: MTHFR C677T approximately doubles the risk of CP in preterm infants. A combination of homozygous MTHFR C677T and heterozygous PGM increases the risk of quadriplegia 5-fold at all gestational ages.

Cerebral palsy (CP) is a disorder affecting 2 to 2.5 children in every 1000 births worldwide. Notwithstanding major improvements in perinatal medicine, the incidence of CP has not decreased over the last 40 years.[1] Research is needed into the causation of CP and the possibilities for its prevention.

A possible association between thrombophilia and CP has been the focus of several small studies. In 1998, it was proposed that both inherited and acquired thrombophilias of the mother and/or the fetus may be responsible for thrombosis in the maternal and/or fetal circulation, resulting in adverse pregnancy outcomes such as CP.[2] There have been 6 small studies or case reports regarding inherited thrombophilic polymorphisms and the subsequent development of CP.[1,3-7] Of these 6 studies, only Smith et al[7] did not find an association between thrombophilia and CP. The only case-control study demonstrated that 20 of 31 cases had surrogate indices for thrombophilia, compared with 2 of 65 controls.[1] The studies by Thorarensen et al,[3] Harum et al,[4] and Steiner et al[5] were case reports, with sample sizes of 3, 1, and 1, respectively. Finally, Halliday et al[6] found evidence of thrombophilia in 5 of 55 cases of CP.

This population-based study is the first large case-control study of CP and hereditary thrombophilias. We compared the prevalence of thrombophilic polymorphisms, common in white populations, in different types of CP cases at different gestational ages and in controls. The thrombophilic polymorphisms studied were: factor V Leiden (FVL), prothrombin gene mutation G20210A (PGM), and methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms C677T and MTHFR A1298C.

Material and methods

Patient selection

The study population comprised all children with CP born in 1986 through 1999 in South Australia (SA) to white mothers (n = 443), ascertained by the SA Cerebral Palsy Register from notifications by pediatricians, hospitals, and child treatment centers. There were 883 infants born to white mothers between the years 1986 and 1999 who were selected as controls for the CP cases. Newborn screening cards were identified by the SA Newborn Screening Program for each case. Potential controls were selected as the 4 infants whose screening cards were filed (by date of receipt) as closely as possible before (n = 2) and after (n = 2) the cards of cases. The dates of birth of the controls were within a few days of the case, the hospital from which the screening cards were taken was of the same category (metropolitan teaching, metropolitan private or country), and samples were taken on approximately the same day of life as the cases, to achieve similarity in condition of the cards. The control population included a higher proportion of preterm infants than the general population, as many of the cases of CP were born preterm and had been referred to metropolitan teaching hospitals. Linkage was attempted for all cases and potential controls to the SA Perinatal Data Collection of births, with its large number of sociodemographic and clinical variables, using Automatch Probabilistic Record Linkage Version 4.3 computer software (MatchWare Technologies Inc, Kennebunk, ME). This was successful for all cases and 1691 controls. 268 (15.8%) of these 1691 controls were excluded as they were children of nonwhite mothers (n = 102); children who had a birth defect as identified from the SA Birth Defects Register (n = 161), or children who died in the first year of life (n = 37) to ensure that they were not potentially cases of CP. Some controls were excluded for more than 1 reason. Two controls were then selected from the remaining controls in each group of 4, using random numbers, to form the control population of 886. As 3 controls had inadvertently been selected more than once, the final number of controls was 883. The data from all cases and controls were de-identified before testing for polymorphisms and statistical analysis. All testing for polymorphisms was undertaken with blinding to case/control status.

This research was approved by the Research Ethics Committee of the Women's and Children's Hospital.

DNA isolation and amplification

Four common polymorphisms associated with thrombophilia were screened for all subjects: FVL (G1691A), PGM (G20210A), MTHFR (C677T), and MTHFR (A1298C). DNA was isolated from the newborn screening cards with the use of the InstaGene Dried Blood Kit (BioRad, Hercules, CA) or the Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

The polymorphic regions were amplified as four DNA fragments, ranging in size from 95 to 120 base pairs (bp), in a single multiplex polymerase chain reaction (PCR). After PCR amplification, the polymorphic base in each gene was determined with the ABI Prism SNaPshot Multiplex Kit (Applied Biosystems, Inc, Foster City, CA). The products were run on a DNA sequencer (ABI 3700) and results analyzed with Genotyper Version 3.7 software. In each case the locus was identified by primer