Cerebral ultrasound abnormalities in offsprings of women with C677T homozygous mutation in the MTHFR gene: a prospective study

Laura Pogliani, Chiara Cerini, Francesca Penagini, Piergiorgio Duca, Chiara Mameli, Gian Vincenzo Zuccotti

Milano, Italy

Background: Perinatal stroke is a common cause of neurologic disability. Being clinically under-recognized, its true incidence is not known. Maternal thrombophilia is likely to be a predisposing factor. To date, a general consensus for evaluation of babies born to mothers with genetic thrombotic predisposition is missing. This study was undertaken to assess the frequency of cerebral abnormalities in the offsprings of women with homozygous C677T mutation in the MTHFR gene, and to seek for association with additional maternal or pregnancy risk factors.

Methods: Mother-infant pairs were consecutively recruited from October 2006 through February 2013. Neonates underwent a thorough physical examination at birth, and a cerebral ultrasound examination (cUS) was performed within 24 hours of their life. In neonates with major cerebral lesions, a thrombophilia panel test was obtained. Follow-up cUS was performed in babies with major or minor cerebral abnormalities.

Results: Ninety-one neonates (47 males) were enrolled. By cUS, abnormalities were detected in 18 (19.8%) neonates. Twelve neonates were diagnosed with a minor lesion; a major ischemic/hemorrhagic lesion was found in 6 neonates. There were a neat male preponderance and significant associations with a history of suspected miscarriage, maternal coagulation factors gene mutations, and reduced protein S or protein C activity.

Conclusions: Our data confirmed a high incidence of cerebral abnormalities in neonates born to women with C677T homozygous mutation in the MTHFR gene. cUS at birth proved to be an effective screening tool or a diagnostic test, that should be routinely performed in babies born to mothers with known thrombotic predisposition.

Key words: cerebral ultrasound; maternal thrombophilia; methylenetetrahydrofolatereductase polymorphism; perinatal stroke

Introduction

Perinatal stroke (PS), an acute neurologic syndrome due to cerebral injury of vascular origin, is a common cause of neurologic disability. The incidence of PS is varied with a frequency ranging from 1 in 4000 live births[1] to an incidence as high as 17% in autopsy studies of term newborns.[2] The broad range of estimates relates to the definition, population enrolled, type of studies that have derived such data, and ascertainment of cases.

By convention, PS encompasses cerebrovascular events occurring between 20 weeks of fetal life and 28 postnatal days,[3] however some authors used a narrower definition (i.e. from 28 weeks of gestation to 7 or 28 days of life).[4,5] Notably, the age at stroke cannot be established with any degree of certainty and can only be conjectured to have occurred in the above mentioned time period. The major clinical-anatomic subtypes comprised: i) arterial ischemic stroke; ii) hemorrhagic stroke; and iii) cerebral sinovenous thrombosis.

Most often, symptomatology is nonspecific and neonates may remain clinically asymptomatic until several months of age, when seizures or signs of motor or cognitive impairment are first noted.[6,7] Thus, the final or presumed diagnosis of PS is often delayed.
Remarkably, in settings with high frequency of neuroimaging, the incidence of PS has been reported to be one in 2300 live births, considerably higher than the incidence in older children, estimated at 3.3 in 100 000 per year in individuals of less than 15 years old. The etiological factors responsible for perinatal stroke are various and diverse for each type of vascular lesion.

Even though a clear embolic source is rarely identified, thromboembolism is considered a leading cause of perinatal cerebral infarction. Among genetic predisposing conditions for spontaneous stroke, increased lipoprotein "a" [Lp(a)] levels, the coagulation factor V Leiden (mutation G1691A), the prothrombin G20210A variant, the methylenetetra-hydrofolatereductase (MTHFR) CT677T genotype, and deficits in protein C or S, are widely described in symptomatic children and adolescents. Conversely, the role of the mentioned prothrombotic conditions in PS is debated.

As in adults and in children >6 months, the increased Lp(a) concentration is the most important risk factor. Deficiencies in protein C or S activity, heterozygosity for factor V Leiden and prothrombin 20210, homozygosity for C677T or compound heterozygosity for the C677T/A1298C alleles in the MTHFR gene, the 4G polymorphism of the plasminogen activator inhibitor 1, and the presence of anti-phospholipid antibodies have also been reported. Additionally, pregnancy, placental and/or maternal disorders might be predisposing conditions for PS.

Pregnancy is physiologically hampered by a hypercoagulable state and pregnant women often have reduced levels of protein S and elevated levels of factor V, factor VIII and fibrinogen. On the other hand, newborn infants are at risk of developing vitamin K deficiency, leading to reduced activity of vitamin K-dependent coagulation factors (i.e. factor II, VII, IX, and X and the Glu-proteins C and S) and abnormal prothrombin levels, thus impairing blood clot formation. Case series and case-control studies suggest that anti-phospholipids syndrome, pre-eclampsia, diabetes, placental thrombosis or abruptio, prolonged rupture of membranes, chorioamnionitis, intra-partum maternal fever, smoking during pregnancy, cocaine abuse, and history of infertility are risk conditions for perinatal stroke.

Placental infarction and subsequent embolization have been proposed as the major causative events: thrombi arising in the placental veins may reach the fetal cerebral circulation through the normally patent foramen ovale and ductus arteriosus. A higher incidence of cerebro-vascular accidents has been described in infants born to mothers with genetic prothrombotic disorders, including polymorphism for the MTHFR gene, factor V Leiden, and prothrombin gene mutation. Recently, we found an intriguing association between maternal homozygous C677T mutation in the MTHFR gene and the occurrence of major ischemic/hemorrhagic cerebral lesions in the offspring.

To date, however, no studies have prospectively evaluated the incidence of major or minor sonographic cerebral abnormalities in infants born to mothers with ascertained genetic susceptibility to thrombosis. Most studies investigating PS are retrospective and limited by small size or referral bias. Additionally, while guidelines for management of pregnant women with documented thrombophilia are available, a general consensus as to the basic evaluation of babies born to mothers with known thrombotic risk factors is missing.

The purpose of this study was to prospectively evaluate the incidence of major and minor cerebral abnormalities by cerebral ultrasound (cUS) in neonates born to mothers with homozygous MTHFR C677T mutation and to evaluate the relationship with maternal or pregnancy conditions predisposing to thrombotic events.

Methods
Study population
Eligible for the present study were neonates born to mothers with MTHFR C677T homozygous mutation, detected as part of thrombophilic screening tests performed in pregnant women with history of hypertension, preeclampsia, recurrent spontaneous abortions, abruptio placenta or intrauterine death, referred to the L. Sacco Hospital High-risk Pregnancy Ambulatory and Birth Center. Given the intrinsic risk of fetal neurologic impairment, exclusion criteria were: maternal substance abuse during pregnancy, multiple pregnancy, severe prematurity (gestational age <32 weeks), very low birth weight (<2SD for gestational age), congenital infection, perinatal asphyxia, chromosomal syndromes, neonatal sepsis, birth related trauma, the need for resuscitation. The study was approved by the L. Sacco Hospital Ethical Commitee. Written informed consent was obtained at birth from parents or legal guardians of the newborn.

Procedures
In the pregnant women, laboratory tests included DNA sequencing for three common gene mutations MTHFR C677T, the missense mutation R506Q in factor V (factor V Leiden) and prothrombin 20210G>A, detected by polymerase chain reaction and analysis of restriction