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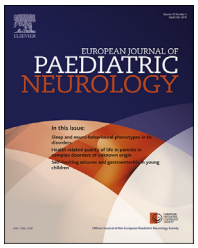

Original Article

The association between antioxidant enzyme polymorphisms and cerebral palsy after perinatal hypoxic-ischaemic encephalopathy

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ABSTRACT

Background: Hypoxic-ischaemic perinatal brain injury leads to the formation of reactive oxygen species (ROS) and the resultant cell and tissue damage may cause neurological sequelae such as cerebral palsy and/or epilepsy. A decrease in the capacity for defending against ROS may increase the susceptibility to cerebral palsy. The aim of this study was to investigate the impact of common functional polymorphisms in the antioxidant genes *SOD2*, *GPX1* and *CAT*, associated with a decreased capacity for defending against ROS, in patients with perinatal hypoxic-ischaemic encephalopathy (HIE).

Methods: 80 patients previously diagnosed with perinatal HIE were included. Genomic DNA was isolated from buccal swabs and genotyped for *SOD2* rs4880, *GPX1* rs1050450 and *CAT* rs1001179 using real-time PCR-based methods.

Results: Among patients with neonatal HIE, carriers of at least one polymorphic *CAT* rs1001179 T allele were significantly associated with development of cerebral palsy compared to non-carriers (univariate logistic regression, $p = 0.026$; OR = 3.36; 95% CI = 1.16–9.76). This difference remained statistically significant after accounting for prematurity. The investigated *SOD2* and *GPX1* polymorphisms were not associated with cerebral palsy after perinatal HIE.

Conclusion: *CAT* rs1001179 polymorphism could be used to identify children that have a higher susceptibility to cerebral palsy after perinatal HIE.

1. Introduction

Severe perinatal hypoxic-ischaemic encephalopathy (HIE) is the most common cause of long-term neurological deficits such as cerebral palsy and/or intellectual disability.[1] Perinatal asphyxia leads to a cascade of neurotoxic events involving energy failure, ultimately resulting in the accumulation of reactive oxygen species (ROS).[2,3] The cell and tissue damage in

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the immature brain with its lower capacity for defending against ROS, in particular lower glutathione peroxidase activity, may eventually cause permanent neurological sequelae such as cerebral palsy and/or epilepsy.[1,4,5] Several antioxidant mechanisms protect cells against oxidative damage. The first line of defence against ROS are the antioxidant enzymes manganese superoxide dismutase (SOD2), glutathione peroxidase 1 (GPX1) and catalase (CAT), which detoxify the superoxide anion and hydrogen peroxide.[6] Their activity and ability to protect cells and tissues from ROS and their damaging products are influenced by functional genetic polymorphisms in antioxidant genes.[7] The *SOD2* rs4880 polymorphism changes the amino acid sequence in the mitochondrial leading sequence (p.Ala16Val), resulting in lower MnSOD activity.[8] *GPX1* rs1050450 polymorphism leads to substitution of p.Pro198Leu by the Leu variant, which is less active than its Pro counterpart.[9] The *CAT* rs1001179 (c.−262 C > T) polymorphism alters the transcription factor binding site in the promoter region, with the polymorphic T allele leading to enhanced gene transcription,[10] however the association with enzyme activity is more complex.[11,12]

The aim of our study was to assess whether common functional antioxidant enzyme polymorphisms are associated with development of cerebral palsy after neonatal HIE.

2. Materials and methods

The study included patients previously diagnosed with perinatal HIE who were consecutively recruited at their regular outpatient follow-up visits to the Department of Child, Adolescent and Developmental Neurology at the Children's Hospital in Ljubljana during the period 2011–2014. All patients were Central European Caucasians. The medical records of all patients were studied retrospectively. The inclusion criterion was documented neonatal HIE grades II–III according to the Sarnat and Sarnat Classification.[13] The exclusion criteria were HIE grade I and the presence of any medical condition that may itself lead to cerebral palsy or neonatal brain disorder (like IUGR, congenital heart disease, brain malformation, genetic or any disorder that may be associated with neonatal encephalopathy and/or later CP as postpartum serious disease – RDS, meconium aspiration, sepsis, neuromuscular disease etc.). Only babies, that were expected to be healthy neonates, but suffered an episode of acute hypoxia during labour with Apgar score of <5 at 5 min and 10 min, foetal umbilical artery pH <7.0, or base deficit ≥12 mmol/L, or both were included in this study. The Sarnat and Sarnat Grading Scale was used as it remains a valid tool for clinical assessment of HIE for both clinical and research purposes in the neonatal period.[13,14] Prematurity was classified according to WHO[15]: patients born between 32 and 37 weeks of gestational age were moderately to late preterm, between 28 and 32 very and under 28 weeks of gestational age extremely premature. Severe prematurity was defined as patients who were born before 32 weeks of gestational age.

The study was approved by the Republic of Slovenia National Medical Ethics Committee and informed consent was obtained from all the participants and/or their parents or legal guardians before inclusion in the study.

Buccal swabs were obtained from all participants and DNA was isolated using the DNA Mini Kit (QIAGEN, Hilden, Germany) according to the protocol recommended by the manufacturer. Genotyping of *SOD2* rs4880 (p.Val16Ala) and *GPX1* rs1050450 (p.Pro200Leu) polymorphisms was performed using TaqMan single nucleotide polymorphism genotyping assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions on the Applied Biosystems ABI 7500 Real Time PCR system (Foster City, CA, USA) as previously described.[16] Genotypes of *CAT* rs1001179 (C. −262 C > T) were determined using KASPar assay (KBiosciences, Herts, UK) according to the published recommended protocol.[17] Due to non-amplification and/or low amounts of DNA, genotypes could not be determined for *SOD2* in four patients, for *GPX1* in three patients and for *CAT* in four patients.

A dominant model, in which heterozygotes and homozygotes for the rare allele were pooled together and compared with the homozygote for the wild-type allele, was used in all analyses. The influence of polymorphisms on CP susceptibility was assessed by logistic regression where odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Statistical analysis was performed using IBM SPSS Statistics, version 19.0 (IBM Corporation, Armonk, NY, USA). A p value of <0.05 was considered statistically significant.

Using Power and Sample Size Calculation program version 3.0.43,[18] we determined the effect size we could detect with 80% power based on the minor allele frequency of each investigated polymorphism. With our available sample size we were able to detect with 80% power ORs above 3.80 for *GPX1*, above 3.85 for *SOD2* and above 3.95 for *CAT*.

3. Results

80 patients with neonatal HIE grades II and III, 39 (48.8%) boys and 41 (51.3%) girls, aged 7 (5–14) years, were included in the study. Among all patients with HIE, 64 (80.0%) developed epilepsy and 51 (63.8%) had CP at follow up. Among 41 (56.2%) preterm children, 19 (26.0%) were moderately to late preterm, 14 (19.2%) very and 8 (11.0%) extremely premature. In the subgroup of patients with CP, 31 (64.6%) were born before 37 weeks gestational age, with prematurity being the most significant clinical factor associated with CP. Children born preterm had significantly higher susceptibility to CP ($p = 0.010$; OR = 3.88; 95% CI = 1.39–10.83) The same trend was observed in patients with severe prematurity ($p = 0.081$; OR = 2.98; 95% CI = 0.88–10.11). The clinical characteristics of all patients are shown in Table 1.

Genotype frequencies of the investigated polymorphisms in genes coding for antioxidant enzymes were compared in patients with and without CP as shown in Table 2.

SOD rs4880 and *GPX* rs1050450 polymorphisms were not significantly associated with the risk of CP ($p = 0.816$ and $p = 0.588$, respectively), while the polymorphic *CAT* rs1001179 allele demonstrated a statistically significant association with the presence of CP at inclusion ($p = 0.026$). Patients with at least one polymorphic *CAT* rs1001179 T allele were more prone to develop CP (OR = 3.36; 95% CI = 1.16–9.76) compared to non-carriers. After adjustment for prematurity, the *CAT* rs1001179 polymorphism remained statistically significantly