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Antioxidant polymorphisms do not influence the risk of epilepsy or its drug resistance after neonatal hypoxic-ischemic brain injury

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ABSTRACT

Purpose: The aim of this study was to investigate if common functional antioxidant polymorphisms are associated with epilepsy after neonatal hypoxic-ischemic encephalopathy (HIE). The antioxidant enzymes manganese superoxide dismutase (SOD2), glutathione peroxidase 1 (GPX1) and catalase (CAT) represent the primary defence mechanism against reactive oxygen species (ROS). Evidence suggests that genetic variants in antioxidant enzymes could influence susceptibility to epilepsy, but to date the relationship between them remains unclear.
Methods: The study comprised 214 patients with epilepsy (64 with and 150 without neonatal HIE) as well as 95 healthy controls. Genomic DNA was isolated from buccal swabs or venous blood samples and genotyped for *SOD2* rs4880, *GPX1* rs1050450 and *CAT* rs1001179 using real-time PCR-based methods.
Results: The investigated polymorphisms influenced neither the overall risk of epilepsy nor the risk of epilepsy after HIE in comparison with healthy controls. Furthermore, no significant difference in genotype distribution was observed between patients with drug-resistant epilepsy and patients in remission in either the group with epilepsy but without HIE or in the group with epilepsy and HIE, although the frequency of drug-resistant cases was higher in the latter group ($p = 0.009$, OR = 2.52; 95% CI = 1.22–4.15).
Conclusion: According to this study, common *GPX1*, *SOD2* and *CAT* polymorphisms do not influence the overall risk of epilepsy after HIE and its drug resistance.

1. Introduction

Hypoxic-ischemic encephalopathy (HIE) develops as a consequence of intrauterine or perinatal hypoxia associated with a variety of maternal, placental and/or foetal conditions [1]. Perinatal asphyxia—oxygen deficit at delivery [2] leads to a cascade of neurotoxic events involving energy failure and the accumulation of reactive oxygen species (ROS) [3,4]. In the immature brain with its reduced capacity for defence against ROS, in particular due to lower glutathione peroxidase activity, the resultant tissue damage may lead either to 'minimal brain damage disorders' (attention deficits and hyperactivity) or to more severe neurological lifelong pathologies such as epilepsy and cerebral palsy (CP) or both [2,5–7].

Antioxidant enzymes represent an important defence mechanism against ROS. It has been suggested that generation of oxidative stress within the brain tissue might play an important role in epileptogenesis [8], and several studies have investigated whether antioxidant enzyme activity influences either susceptibility to epilepsy or its characteristics such as drug resistance [9,10].

Antioxidant enzymes such as manganese superoxide dismutase (SOD2), glutathione peroxidase (GPX1) and catalase (CAT) detoxify superoxide anion and hydrogen peroxide and constitute the primary defence against ROS. The activity of these antioxidant enzymes protects cells from ROS and is influenced by functional genetic polymorphisms. *GPX1* rs1050450 polymorphism leads to p.Pro198Leu substitution with the Leu variant being less active than its Pro counterpart [11]. *SOD2* rs4880 polymorphism alters the amino acid in the mitochondrial leading sequence (p.Ala16Val) resulting in lower MnSOD activity [12]. The *CAT* rs1001179 (c.-262 C > T) polymorphism alters the transcription factor binding site in the promoter region [13], with the polymorphic T allele leading to enhanced gene transcription, but its association with enzyme

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activity is more complex [14,15]. The aim of this study was to investigate if functional polymorphisms in antioxidant genes contribute to susceptibility to epilepsy after neonatal HIE and/or its drug-resistance.

2. Methods

2.1. Participants

Two hundred and fourteen patients—children and adolescents of Slovenian Central European Caucasian origin with epilepsy were consecutively recruited during their regular outpatient follow-up visits in our hospital during the period January 2011–December 2014. The medical data of all patients were collected retrospectively from electronic and/or paper records. In particular, data from the neonatal period regarding the level of consciousness, activity, neuromuscular control, complex/primitive reflexes, autonomic functions in a newborn and the presence of seizures were reviewed. The patients were divided into two groups according to the aetiology of their epilepsy. The inclusion criterion for the first group was documented neonatal hypoxic-ischemic encephalopathy (HIE) grades II–III, according to the Sarnat and Sarnat classification [16]. This group has been a part of our previous research [17]. The Sarnat and Sarnat Grading Scale was used, as it represents a valid tool for clinical assessment of HIE for both clinical and research purposes in the neonatal period [16,18]. Only neonates, that were expected to be healthy newborns, but suffered an episode of acute perinatal hypoxia with Apgar score <5 at 5 min, foetal umbilical artery pH <7.0 were included in the first study group. Perinatal, neonatal risk factors and cranial US scans, which were a part of routine clinical care, were reviewed, while neonatal MRI brain scans (adjusted for age in preterms) were not performed as a routine clinical work in one third of patients. The exclusion criteria for the first group were HIE grade I and the presence of any other concurrent medical condition that may itself lead to the development of epilepsy (intrauterine growth retardation, neonatal sepsis, congenital heart disease, brain malformation, genetic, metabolic or any other disorders that may be associated with neonatal encephalopathy). Patients with insufficient data regarding perinatal history were also excluded.

The inclusion criterion for the second group was a diagnosis of epilepsy regardless of the cause, but with no perinatal HIE (i.e. documented normal pregnancy and perinatal data). Collected clinical data for all patients consisted of gender, date of birth, gestational age, aetiology of epilepsy and the responsiveness of seizures to drug treatment. The latter was classified as epilepsy in remission if no seizures occurred in the last year, or as drug-resistant epilepsy if at least one seizure occurred in the last year of the follow up.

The control group consisted of 95 healthy Slovenian blood donors younger than 30 years of age at the time of blood sampling. The study was approved by the Republic of Slovenia National Medical Ethics Committee and written informed consent was obtained from all the participants and/or their parents or legal guardians prior to inclusion in the study.

2.2. DNA isolation and genotyping

Venous blood samples or buccal swabs were obtained for DNA extraction. Samples of 3 ml blood were collected from 150 participants during their regular outpatient visits. DNA was isolated using the FlexiGene DNA Kit (Qiagen, Hilden, Germany). Buccal swabs were obtained from 64 patients who did not need regular blood control. The DNA Mini Kit (QIAGEN) was used for DNA extraction from buccal swabs according to the recommended protocol. DNA from healthy controls was also isolated from venous blood using the FlexiGene DNA Kit (Qiagen). For *GPX1* rs1050450 and *SOD2* rs4880, genotyping was performed using TaqMan genotyping assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions as previously described [19]. Genotypes of *CAT* rs1001179 were determined using KASPar assay (KBiosciences, Herts, UK) according to the recommended protocol [20].

The frequencies, median and the interquartile range were used to describe the distribution of categorical and continuous variables, respectively. To assess deviation from Hardy–Weinberg equilibrium (HWE), a standard chi-square test was used. A dominant model was used in all analyses. To assess the effect of polymorphisms on susceptibility to epilepsy and drug resistance, we calculated odds ratios (ORs) and 95% confidence intervals (95% CI) using logistic regression analysis. Statistical analysis was performed using IBM SPSS Statistics, version 19.0 (IBM Corporation, Armonk, NY, USA). A $p < 0.05$ or less was considered statistically significant.

To determine the effect size that we could detect based on the minor allele frequency of investigated polymorphisms, we used the Power and Sample Size Calculation programme version 3.0.43 [21]. In the case-control analysis, we were able to detect with 80% power ORs above 2.13 for *GPX1*, above 2.01 for *SOD2* and above 2.07 for *CAT*.

3. Results

The overall study population included 214 patients with epilepsy, 64 (29.9%) with HIE and 150 (70.1%) with other causes of epilepsy and no HIE. Baseline characteristics of all patients are presented in Table 1. Of the 95 healthy controls, 46 (48.9%) were male and the median age was 21 (20–23) years.

Table 1
Clinical characteristics of the study population (N = 214).

Variable		All patients with epilepsy N = 214 N (%)	Epilepsy with HIE N = 64 N (%)	Epilepsy without HIE N = 150 N (%)
Gender	Male	99 (46.3)	32 (50.0)	67 (44.7)
	Female	115 (53.7)	32 (50.0)	83 (55.3)
Age at recruitment (years) Median (25–75%)		10 (6–18)	7 (4–14)	11 (3–30)
HIE	Yes	64 (29.9)	64 (100.0)	–
	No	150 (70.1)	–	150 (100.0)
CP at follow-up		47 (22.0)	41[b] (64.1)	6 (4.0)
Patients in remission[a]		105 (49.5)	22 (35.5)	83 (55.3)
Patients with drug-resistant epilepsy[a]		107 (50.5)	40 (64.5)	67 (44.7)

HIE = hypoxic-ischemic encephalopathy; CP = cerebral palsy.
[a] Data on remission missing for two patients.
[b] Data on cerebral palsy missing for two patients.