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ORIGINAL PAPER

Variants of the *OLIG2* Gene are Associated with Cerebral Palsy in Chinese Han Infants with Hypoxic–Ischemic Encephalopathy

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Abstract

Cerebral palsy (CP) is a leading cause of neurological disability among young children. Congenial and adverse perinatal clinical conditions, such as genetic factors, perinatal infection, and asphyxia, are risk factors for CP. Oligodendrocyte transcription factor (OLIG2) is a protein that is expressed in brain oligodendrocyte cells and is involved in neuron repair after brain injury. In this study, we employed a Chinese Han cohort of 763 CP infants and 738 healthy controls to study the association of *OLIG2* gene polymorphisms with CP. We found marginal association of the SNP rs6517135 with CP ($p=0.044$) at the genotype level, and the association was greatly strengthened when we focused on the subgroup of CP infants who suffered from hypoxic–ischemic encephalopathy (HIE) after birth, with $p=0.003$ (OR = 0.558) at the allele level and $p=0.007$ at the genotype level, indicating a risk-associated role of the T allele of the SNP rs6517135 under HIE conditions. The haplotype CTTG for rs6517135–rs1005573–rs6517137–rs9653711 in *OLIG2* was also significantly associated with the occurrence of CP in infants with HIE ($p=0.01$, OR = 0.521). Our results indicate that in the Han Chinese population, the polymorphisms of *OLIG2* were associated with CP, especially in patients who had suffered HIE injury. This finding could be used to develop personalized care for infants with high susceptibility to CP.

Keywords Cerebral palsy · *OLIG2* · HIE · SNP · Hypoxia · Ischemia

Introduction

After the successful control of poliomyelitis in most countries, cerebral palsy (CP) has become the leading cause of physical disability among children, affecting approximately

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1 in 500 live births and 4–10 in 100 live births with less than 28 weeks of gestation (Wimalasundera and Stevenson 2016). CP is not a specific disease but a group of movement and posture disorders that are accompanied by disturbance of sensation, cognition or other brain functions (Downs et al. 2018). CP is caused by non-progressive injuries in developing brains (Bax et al. 2005). Ninety percent of the injuries are believed to occur during the prenatal and perinatal periods (Graham et al. 2016), including hypoxia–ischemia and infections. These adverse factors usually cause neuron and glial cell death, leading to neonatal encephalopathies, such as periventricular leukomalacia (PVL) and hypoxic–ischemic encephalopathy (HIE) (Baburamani et al. 2012; Xie et al. 2016). HIE affects 1.5 infants per 1000 live births in developed countries (Kurinczuk et al. 2010), and usually results from perinatal asphyxia that causes brain cell death via excitotoxicity, inflammation, and oxidative stress (Northington et al. 2011; Strunk et al. 2014; Thornton et al. 2017). HIE remains an important cause of neonatal death, and in survivors, HIE frequently leads to significant long-term disabilities, such as CP, mental retardation (MR), epilepsy, and other neurophysiological handicaps (Fathali et al. 2010). Moderate to severe HIE is associated with severe disability in 25% of survivors and a mortality rate of 15–25% (Edwards et al. 2010; Shankaran 2012). Hypothermia and erythropoietin have been shown to reduce neurological disability in cases of moderate and severe HIE (Liu et al. 2017; Zhu et al. 2009). In addition to neonatal encephalopathy, in recent years, an increasing number of genetic factors, such as IL-6 (Bi et al. 2014), ATG5 (Xu et al. 2017), ADD3 (Kruer et al. 2013), and EAAT2(Rajatileka et al. 2018), have been found to participate in, modulate, and even induce the pathological process of CP(van Eyk et al. 2018).

The white matter of the brain contains oligodendrocytes (OLs), which are a type of neuroglia that form myelin sheaths around neuronal axons and are essential for the propagation of nerve impulses. OLs are vulnerable cells in developing brains. Brain injury induces great loss of OLs, after which efficient regeneration of OLs from their progenitors is pivotal for neuron repair and protection from CP (Meberg and Broch 2004). Oligodendrocyte transcription factor (OLIG2), a protein that is expressed in oligodendrocyte progenitor cells (OPCs), influences the repair process (Gaber and Novitch 2011). OLIG2 is a basic helix-loop-helix transcription factor that first determines motor neuron differentiation in early embryonic brains (Mie et al. 2012; Sagner et al. 2018) and subsequently directs the proliferation and differentiation of OPCs in developing or injured brains (Buffo et al. 2005; Zuo et al. 2018). Malfunctioning of OLIG2 has detected in brain cancer (Kosty et al. 2017; Tsigelny et al. 2016) and Down syndrome (Lu et al. 2012). A number of genetic studies have also been conducted to explore the link between *OLIG2* and various neurological and psychiatric diseases. A direct association between the *OLIG2* single nucleotide polymorphism (SNP) rs1059004 and reduced white matter integrity was identified in healthy volunteers in the UK (Prata et al. 2013). A family-based genetic association study of *OLIG2* found positive associations between the gene and obsessive–compulsive disorder (Stewart et al. 2007). This gene has also been linked to schizophrenia in both European and Chinese populations (Georgieva et al. 2006; Huang et al. 2008). Given the important role that OLIG2 plays in the central nervous system and potentially in the pathology of CP, we aimed to explore the genetic relevance of OLIG2 in CP by performing a case-control study in the Chinese Han population.

Materials and Methods

Subjects

The subjects were unrelated Chinese Han children recruited from the centers for CP rehabilitation in the Third Affiliated Hospital of Zhengzhou University and Zhengzhou Children's Hospital. The study population consisted of 763 CP cases (235 girls and 528 boys ranging in age from 5 months to 61 months, with a mean age of 18.28 months) and 738 healthy controls (248 girls and 763 boys ranging in age from 5 to 120 months, with a mean age of 19.47 months). The two groups were studied during the same period and matched for age, gender and ethnicity. Using the power analysis program G-Power, the statistical power of this study in the detection of a significant association with an effect size index of 0.1 was 0.97.

Child neurologists diagnosed and classified CP by clinical examination and analysis of medical records, including brain imaging records, according to the guidelines proposed by the "Surveillance of CP in Europe" network (Sellier et al. 2010). The controls had no familial relationships with the CP patients and did not exhibit neurological or psychological symptoms. Children in either the CP or control group with myopathy, genetic syndromes, chromosomal anomalies, or metabolic anomalies were excluded. The demographics of the participants are summarized in Supplementary Table 1. In addition, all of the 763 CP patients and 444 of the 738 healthy controls also had detailed medical records. To evaluate the risks associated with the clinical conditions for CP and to better characterize our study cohort, an epidemiological association analysis was conducted with these records.

Written informed consent was obtained from the parents of the participants in this study after providing a detailed explanation of the procedure. The research protocol was reviewed and approved by the ethics committee of Zhengzhou University and the Medical Academy of Henan

Province (201201002) in accordance with the Declaration of Helsinki.

Medical Records

The database of medical records for this study contains information on the risk factors associated with CP, which can be categorized into maternal factors and neonatal factors.

The maternal factors include age of pregnancy, history of infertility, history of abortion, pregnancy-induced hypertension (PIH), threatened preterm labor (TPL), premature rupture of membrane (PROM), placental abruption, abnormal amniotic fluid, and single or multiple births. The neonatal factors include birth weight, gestation age, birth asphyxia, coiling of the umbilical cord, intrauterine growth retardation (IGR), and intracranial hemorrhage (ICH). In addition to these risk factors, complications concomitant with CP were also recorded, including PVL, HIE, MR, and language handicap.

PIH was diagnosed and classified according to the criteria recommended by the American Congress of Obstetricians and Gynecologists, including a systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure of 90 mmHg or higher on two occasions at least 6 h apart occurring after 20 weeks of gestation in a pregnant woman with previously normal blood pressure and without detectable urinary protein (Sibai and Stella 2009). TPL was diagnosed by the occurrence of at least one of the following incidents during pregnancy: the mother was admitted to the hospital prenatally with an episode of TPL, the mother received antenatal steroids or tocolytics, or a note was made in the delivery room chart that the patients had a history of TPL during pregnancy (Campbell et al. 2012). PROM was diagnosed based on pooling of amniotic fluid in the vagina, amniotic fluid ferning patterns, and a positive nitrazine test (Ferrell et al. 2009). Placental abruption refers to partial or complete peeling of the placenta from the uterine wall after 20 weeks of pregnancy or during childbirth (Xing and Guo 2013). Abnormal amniotic fluid refers to too much or too little amniotic fluid or amniotic fluid contamination at birth. Neonatal birth asphyxia was diagnosed using the criteria of the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists, and newborns were included when at least three of the four criteria were met (Avasiloaiei et al. 2013). Coiling of the umbilical cord was diagnosed by B-ultrasound or visual inspection during childbirth. IGR, also known as intrauterine growth restriction or fetal growth restriction, refers to poor growth of a fetus in the mother's womb during pregnancy, resulting in a low birth weight. All infants with birth weights lower than the 10th percentile or 2 SDs of their average birth weight, based on the intrauterine growth curve (Hou et al. 2009), were identified as exhibiting IGR in this study (Aucott et al. 2004). ICH includes subdural hemorrhage, subarachnoid hemorrhage, periventricular subependymal-intraventricular hemorrhage, cerebellar hemorrhage, and intracerebral hemorrhage. Cases with ICH were identified by the neuroimaging method (Benders et al. 2014). PVL was identified by the presence of parenchymal densities or lucencies around the ventricles during ultrasound examination of the head performed any time after 14 days postbirth (Soraisham et al. 2013) and was further confirmed by MRI. Diagnosis of HIE requires a combination of parameters that are indicative of metabolic acidosis within the first hours after birth. These parameters include low umbilical cord blood pH (<7.0); a base deficit of more than 12 mEq/L; and evidence of a need for respiratory support starting in the first minutes after birth, with low Apgar scores at and beyond 5 min after birth. The Sarnat Grading Scale was used as a classification scale for HIE of the newborn. MR, also termed intellectual disability (Shea 2012), was identified based on a score of less than 70 on the Bayley scales for measurement of the mental development index (Zhu et al. 2009). Language handicap was assessed by the S-S language development assessment scale (CRRC version) and articulation disorder assessment scale.

Genotyping

Genomic DNA was extracted from venous blood using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA). A total of six SNPs with minor allele frequencies greater than 0.1 in the Chinese Han population were selected from the dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/) and the phase II genotyping data of the HapMap project (http://www.hapmap.org). The six SNPs were rs6517135, rs1005573, rs146665636, rs1059004, rs6517137 and rs9653711, with rs6517135 located upstream of *OLIG2*, rs1005573 in the intron, rs146665636 in the exon (synonymous), rs1059004 and rs6517137 in the 3′ UTR region, and rs9653711 downstream of *OLIG2*. Probes and primers were designed by using the Sequenom online tools (https://www.mysequenom.com). After amplification of polymorphism-spanning fragments by multiplex PCR, genotyping was performed with the Sequenom MassARRAY SNP genotyping platform (Sequenom, San Diego, CA, USA). SpectroTYPER software (Sequenom, Inc.) was used for genotype calling.

Statistical Analysis

For epidemiological comparison of medical records between the CP and control groups, because the parameter of age was not balanced between the two groups ($p=0.025$, data

Table 1 Evaluation of the associations of clinical maternal and neonatal factors with cerebral palsy

Factors	CP group (%)	Control group (%)	*p* value	Odds ratio (95% CI)
Maternal factors				
Age of pregnancy			**0.013**	1.43 (1.08–1.91)
> 30 years old	194 (26.3)	89 (20.1)		
≤ 30 years old	545 (73.7)	354 (79.9)		
Unknown	24	1		
Multifetal pregnancy			0.381	0.68 (0.28–1.62)
Yes	11 (1.4)	10 (2.3)		
No	750 (98.6)	433 (97.7)		
Unknown	2	1		
Infertility history			0.609	1.25 (0.53–2.92)
Yes	17 (2.4)	8 (1.8)		
No	702 (97.6)	435 (98.2)		
Unknown	44	1		
Abortion history			**0.007**	1.618 (1.14–2.30)
Yes	130 (17.3)	50 (11.3)		
No	620 (82.7)	391 (88.7)		
Unknown	13	3		
PIH			**0.038**	2.59 (1.05–6.35)
Yes	26 (3.4)	6 (1.4)		
No	737 (96.6)	437 (98.6)		
Unknown	0	1		
TPL			0.728	1.09 (0.69–1.72)
Yes	57 (7.5)	30 (6.8)		
No	705 (92.5)	413 (93.2)		
Unknown	1	1		
PROM			**0.034**	1.67 (1.04–2.68)
Yes	70 (9.2)	25 (5.6)		
No	693 (90.8)	418 (94.4)		
Unknown	0	1		
Placental abruption			**0.031**	3.26 (1.11–9.52)
Yes	22 (2.9)	4 (0.9)		
No	741 (97.1)	435 (99.1)		
Unknown	0	5		
Abnormal amniotic fluid			< **0.001**	4.03 (2.73–5.92)
Yes	194 (25.5)	34 (7.8)		
No	566 (74.5)	404 (92.2)		
Unknown	3	6		
Neonatal factors				
Gestation age			**0.002**	3.13 (1.51–6.47)
Preterm	45 (5.9)	9 (2.0)		
Full term	717 (94.1)	434 (98.0)		
Unknown	1	1		
Birth weight			**0.002**	21.89 (2.99–160.40)
< 3500 g	36 (4.7)	1 (0.2)		
≥ 2500 g	727 (95.3)	427 (99.8)		
Unknown	0	16		
Birth asphyxia			< **0.001**	19.12 (10.00–36.58)
Yes	227 (29.8)	10 (2.3)		
No	536 (79.2)	433 (97.7)		
Unknown	0	1		

Table 1 (continued)

Factors	CP group (%)	Control group (%)	*p* value	Odds ratio (95% CI)
Coiling of umbilical cord			**< 0.001**	2.23 (1.56–3.18)
Yes	155 (20.3)	45 (10.2)		
No	607 (79.7)	397 (89.8)		
Unknown	1	2		
IGR			**0.002**	23.80 (3.25–174.22)
Yes	37 (5.0)	1 (0.2)		
No	709 (95.0)	442 (99.8)		
Unknown	17	1		
ICH			**0.002**	21.92 (3.00–160.38)
Yes	37 (4.8)	1 (0.2)		
No	726 (95.2)	442 (99.8)		
Unknown	0	1		

p values < 0.05 are indicated in bold

PIH pregnancy-induced hypertension, *TPL* threatened preterm labor, *PROM* premature rupture of membrane, *IGR* intrauterine growth retardation, *ICH* intracranial hemorrhage

not shown), logistic regressions were employed to evaluate the associations of the clinical factors with CP and simultaneously adjust for age. The regressions were conducted in SPSS (version 22; IBM, USA).

For genetic analysis, the Hardy–Weinberg equilibrium (HWE) test, allele and genotype association analysis, estimation for pairwise linkage disequilibrium (LD), and haplotype association analysis were all performed using the SHEsis online software platform (http://analysis.bio-x.cn/myAnalysis.php). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were provided. A *P*-value < 0.05 was considered statistically significant.

Results

Clinical Risk Factor Evaluation

Based on the detailed medical records for 763 CP cases and 444 controls, the candidate risk factors for CP were categorized into 2 groups, i.e., maternal and neonatal factors (Table 1).

Among the maternal factors, abnormal amniotic fluid was a significant risk factor for CP ($p < 0.001$). Mothers who underwent abortion had an increased likelihood of having babies with CP ($p = 0.005$). In addition, an age of pregnancy greater than 30 ($p = 0.016$), PIH ($p = 0.032$), PROM ($p = 0.028$), and placental abruption ($p = 0.023$) occurred with high frequency in mothers with children who had CP. A history of infertility, multiple births, and TPL were not significantly associated with CP.

Among the neonatal factors, birth asphyxia ($p < 0.001$, OR = 18.34), low birth weight ($p < 0.001$, OR = 21.14), IGR ($p < 0.001$, OR = 23.07), and ICH ($p < 0.001$, OR = 22.53) were found to be significantly associated with CP, and notably, all four factors increased the risk of CP by more than 18-fold (OR > 18). In addition, preterm birth ($p = 0.002$) and coiling of the umbilical cord ($p < 0.001$) were also associated with an increased incidence of CP.

Genetic Association Analysis in All CP Subjects

Among the original six SNPs, the genotype data for the SNP rs146665636 were of low quality (> 10% missing data), and the SNP rs1059004 did not pass the HWE test. Both of the SNPs were excluded, and the other four SNPs (rs6517135, rs1005573, rs6517137, and rs9653711) were analyzed for association. Single-locus association analyses of allelic and genotypic frequencies in each group are presented in Supplementary Table 2. Most of the SNPs were not found to be associated with CP at either the allele or genotype level. Only the SNP rs6517135 exhibited a marginal significance of association with CP ($p = 0.044$) at the genotype level, which disappeared after Bonferroni correction. The LD among the four SNPs was computed and is presented in Supplementary Table 3. The D' ranged from 0.759 to 0.965, indicating a strong LD among these markers. Haplotype analysis was then conducted for these four SNPs. Although there was no overall effect of the haplotypes on CP ($p > 0.05$), the haplotype CTTG for rs6517135–rs1005573–rs6517137–rs9653711 exhibited significant enrichment in the control group, indicating a potential protective effect of this haplotype against CP ($p = 0.02$, OR = 0.78) (Supplementary Table 4).

Table 2 Result of the association analysis with the subgroup of CP subjects with HIE

Group	Minor allele	Allele frequency		*p* value	Odds ratio (95% CI)	Genotype frequency			*p* value	HWE
		C (%)	T (%)			C/C (%)	C/T (%)	T/T (%)		
rs6517135										
CP with HIE	C	32 (0.155)	174 (0.845)	**0.003**	0.558	3 (0.029)	26 (0.252)	74 (0.718)	**0.007**	0.699
Control		357 (0.248)	1083 (0.752)		(0.376–0.829)	36 (0.050)	285 (0.396)	399 (0.554)		0.099
rs1005573										
CP with HIE	T	131 (0.675)	63 (0.325)	0.127	1.281	43 (0.443)	45 (0.464)	9 (0.093)	0.295	0.569
Control		886 (0.619)	546 (0.381)		(0.932–1.763)	267 (0.373)	352 (0.492)	97 (0.135)		0.261
rs6517137										
CP with HIE	C	11 (0.054)	193 (0.946)	0.123	0.609	0 (0.000)	11 (0.108)	91 (0.892)	0.280	0.565
Control		121 (0.086)	1293 (0.914)		(0.323–1.150)	2 (0.003)	117 (0.165)	588 (0.832)		0.127
		C (%)	G (%)			C/C (%)	C/G (%)	G/G (%)		
rs9653711										
CP with HIE	C	31 (0.152)	173 (0.848)	0.282	1.254	0 (0.000)	31 (0.304)	71 (0.696)	0.203	0.070
Control		176 (0.125)	1232 (0.875)		(0.829–1.897)	6 (0.009)	164 (0.233)	534 (0.759)		0.085

p values < 0.05 are indicated in bold

Genetic Association Analysis in the Subgroup of CP Subjects with HIE

Perinatal hypoxia and ischemia are well-known risk factors for CP. The function of the neuronal development protein OLIG2 could be crucial under these adverse conditions. Therefore, we further scrutinized the genetic association of *OLIG2* in the subgroup of CP subjects with HIE, and we found that the SNP rs6517135 exhibited a significant association with this subgroup of children with CP at both the allele and genotype levels ($p = 0.003$ and $p = 0.007$, respectively; Table 2). Moreover, the minor allele C of the SNP rs6517135 exhibited an odds ratio of 0.558 (95% CI 0.376–0.829) in the association analysis, indicating a protective effect of this minor allele C or a risk effect of the major allele T for CP. Haplotype analysis was also conducted for the subgroup of CP subjects with HIE. The haplotype CTTG for rs6517135–rs1005573–rs6517137–rs9653711 maintained its enrichment in the control group with a small *p* value (0.01) and a low OR value (0.521), indicating the protective role of this haplotype in HIE. The overall *p* value for the haplotype analysis was 0.02, which was statistically significant (Table 3).

In addition, we analyzed the associations of the three paresis subtypes of CP (spastic CP, dyskinetic CP and ataxia) and the mixed type of CP with the haplotype CTTG respectively in all CP patients and in CP patients after HIE. The results indicated that the haplotype CTTG was associated with the spastic subgroup in all CP patients ($p = 0.02$) and in the CP patients after HIE ($p = 0.005$) (data not shown). Despite of these associations, CTTG haplotype was in fact distributed across all of these CP subtypes and not found significantly enriched in any specific subtype of CP, therefore this haplotype was far from being a determinant factor for any paresis subtypes.

Discussion

Perinatal Asphyxia-Related Risk Factors are Prominent in Cerebral Palsy

CP is a group of movement and posture disorders caused by non-progressive injury to the developing brain. The etiology of CP varies based on the timing of the specific injury or injuries, approximately 80% of which occur prenatally (Wimalasundera and Stevenson 2016). The risk factors for CP have been extensively studied (McIntyre et al. 2013). Factors such as birth asphyxia and infections are well recognized, among which perinatal asphyxia induced cerebral hypoxia ischemia-related injuries are more prominent (Rocha-Ferreira and Hristova 2016). In this study, we first reevaluated some of these clinical risk factors in a cohort

Table 3 Result of the haplotype analysis with the subgroup of CP subjects with HIE

D'/r2	Case (freq)	Control (freq)	Fisher's p	Pearson's p	Odds ratio (95% CI)
C T C G	9.00(0.048)	98.30(0.072)	0.17	0.17	0.62 [0.31–1.24]
C T T G	18.00(0.096)	220.68(0.162)	**0.01**	**0.01**	0.52 [0.31–0.86]
T C T G	128.00(0.681)	807.40(0.594)	0.10	0.10	1.32 [0.95–1.83]
T T T C	28.00(0.149)	136.05(0.100)	0.07	0.07	1.51 [0.97–2.33]
T T T G	5.00(0.027)	43.44(0.032)	0.63	0.63	0.79 [0.31–2.03]
Total	–	–	**0.02**	**0.02**	–

p values < 0.05 are indicated in bold; All the haplotypes whose frequency was < 0.03 were ignored in analysis; Loci chosen for hap-analysis: rs6517135–rs1005573–rs6517137–rs9653711

of 763 CP patients and 444 controls from Henan province, China. Most of our results were consistent with previous findings, supporting the prevalence of cerebral hypoxia-ischemiarelated risk factors in the antenatal or postnatal period of CP patients (Derrick et al. 2007; Rumajogee et al. 2016). Four neonatal factors exhibited the highest risk for CP with high ORs in this study. Two of these factors, namely, birth asphyxia and intracranial hemorrhage, are directly associated with the hypoxic and ischemic status of the developing brain. Severe birth asphyxia and intracranial hemorrhage are precursors of both HIE and CP (Murabayashi et al. 2008; Shah et al. 2005). The other two factors, namely, low birth weight and IGR, were less frequently linked to CP. Antenatal hypoxia occurs in IGR, suggesting that the supply of oxygen to the fetal brain is as important as that to the brains of newborns. Low birth weight is associated with IGR but can be caused by multiple factors, such as malnutrition. Enhancement of the clinical care provided to babies with low birth weights is recommended by this study. Maternal factors can reflect antenatal in utero conditions (Villamor et al. 2017). The abortion history of mothers was found to be a risk factor in our study. It has been reported that mothers of infants with CP had a relatively high proportions of spontaneous abortions (Pharoah et al. 1987), which could be associated with poor oxygen or energy supply in the uterine environment (Hustin et al. 1990). Moreover, chronic reduction of uterine blood flow was observed in mothers with PIH, which induces IGR (Lang et al. 2003). Both PIH and IGR were revealed as risk factors in our subjects. In addition to the factors mentioned above, other risk factors, such as coiling of the umbilical cord and placental abruption, also indicate a potential shortage of oxygen or blood supply in utero for the baby. Therefore, based on the medical records, it can be concluded that our CP patients were subjected to various pressures associated with perinatal hypoxia and ischemia.

The SNP rs6517135 in the *OLIG2* Gene was Associated with Susceptibility to CP with HIE

We found that the SNP rs6517135 in *OLIG2* was marginally associated with CP patients in general, and the association was greatly strengthened in the subgroup of patients who suffered from HIE. The other two SNPs, namely, rs1005573 and rs9653711, were once found to be associated with schizophrenia (Georgieva et al. 2006) and obsessive–compulsive disorder (Stewart et al. 2007), respectively, but were not found to be associated with CP in our subjects. However, a strong LD (average D'>0.89) was observed among the four SNPs in our cohort, indicating potential interactions between these SNPs.

Both the major allele T and the genotype TT of the SNP rs6517135 were significantly enriched in children with CP and HIE. This SNP (chromosomal position 21:33025263) is located in the promoter region of the *OLIG2* gene, which confers the possibility that this SNP may regulate the risk for HIE and CP by influencing the transcription of the *OLIG2* gene. In the perinatal period, the brain is at the crucial stage of synaptogenesis and myelination of extended axons (Tau and Peterson 2010). Any abbreviated exposure to hypoxia could be sufficient to induce CNS dysmyelination, modulate spinal motor neuron composition, impair motor development (Watzlawik et al. 2015), and cause white matter injury (Zhang et al. 2016). HIE is the most frequent neonatal encephalopathy in infants with CP who are full-term or of normal birth weight. HIE is usually caused by oxygen deprivation of the developing brain and is the most severe form of hypoxia and ischemia in the brains of newborns (Meberg and Broch 2004; Silbereis et al. 2010). Both antepartum and intrapartum factors associated with cerebral hypoxia ischemia can contribute to neonatal HIE (Martinez-Biarge et al. 2013). OLs are a type of glial cell that are essential for myelination and remyelination of neurons after injury (Dizon et al. 2010). Extensive loss of OLs or arrest of OL lineage maturation could also be caused by cerebral hypoxia -ischemia. Therefore, timely proliferation of OLs is crucial for brain recovery after injuries such as HIE (Park et al. 2015; Segovia et al. 2008). OLIG2, a transcription factor

that is widely expressed in the CNS (Ono et al. 2009), aids the proliferation of OLs (Gaber and Novitch 2011; Li et al. 2016) and plays a pivotal role in neural repair (Buffo et al. 2005). It has been found that different phosphorylation states of OLIG2 can regulate the proliferation of neural progenitors (Sun et al. 2011), while enhanced *OLIG2* expression can promote differentiation and migration of OL progenitor cells to facilitate remyelination of neurons (Wegener et al. 2015). Precise timing and control of the expression of OLIG2 is crucial for this process (Mei et al. 2013). Therefore, it is possible that the risk-associated T allele of the SNP rs6517135 in the promoter region of *OLIG2*, which was identified in our study, may hinder the repair ability of OLs by interfering with the expression of *OLIG2* in OLs. As we were unable to measure the expression of *OLIG2* in the brains of the subjects in current study, future studies are encouraged to examine this hypothesis (Mitkus et al. 2008).

In addition, in the 105 CP patients with HIE in our study, there were only 31 (29.5%) and 5 (4.8%) patients with grade II and III HIE, respectively, according to the Sarnat score. More than half (65.7%) of the patients experienced mild HIE but developed CP. Thus, HIE was not necessarily a major cause of brain injury in our cohort but could be a factor that interacts with underlying genetic defects to collectively cause CP. In other words, the genetic risk factor found in this study probably played its role in causing CP under the adverse condition of HIE in our subgroup of CP patients with HIE.

Conclusion

In summary, we investigated 4 SNPs in the *OLIG2* gene locus for association with CP within a Han Chinese population consisting of 763 CP patients and 738 matched controls. We found that the SNP rs6517135 in the promoter region of the *OLIG2* gene was marginally associated with CP patients in general, and the association was greatly strengthened in the subgroup of patients who suffered from HIE before acquiring CP. HIE is a severe form of neonatal cerebral hypoxia-ischemia, which were also found to be the main clinical risk factors for CP in this study. Genetic risk factors can affect neuronal recovery from injuries such as HIE. It is possible that the risk-associated T allele of the SNP rs6517135 in the promoter region of *OLIG2* hinders the repair ability of OLs by interfering with the expression of *OLIG2*. This result needs to be validated by studies with larger sample sizes and in other populations.

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Author Contributions LS analyzed the data and prepared the manuscript. LX and MW collected the clinical data and blood samples. DZ, JS, CM, CG, XZ and YS helped collect the clinical data and blood samples. YW and DB performed the MassARRAY genotyping experiment. QX guided the experiment and data analysis. QX, CZ and XW conceived the study. QX and CZ reviewed and edited the manuscript and gave the final approval for publication of this version of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest. The submitted work was carried out in the absence of any personal, professional or financial relationships that could potentially be construed as a conflict of interest.

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