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The correlation between AGT gene polymorphism and neonatal hypoxic-ischemic encephalopathy (HIE)

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Abstract. – OBJECTIVE: To explore the correlation between the rs2067853 polymorphism in angiotensinogen (AGT) gene and neonatal hypoxic-ischemic encephalopathy (HIE).

PATIENTS AND METHODS: A total of 96 neonatal patients with HIE and 123 healthy neonates were selected. General clinical data were collected and TaqMan-MGB probe method was adopted to detect the rs2067853 polymorphism in angiotensinogen (AGT) gene.

RESULTS: The frequency of advanced maternal age, low maternal age, maternal renal insufficiency, abnormal labor, amniotic fluid contamination and umbilical cord abnormality in the observation group was higher than that in the control group ($p<0.05$), and there was no significant difference between the two groups in the frequency of pregnancy-induced hypertension or eclampsia, maternal anemia, routine prenatal examination, natural childbirth, placental abnormality and abnormal birth weight ($p>0.05$). There was a difference in genotype distribution frequency between the two groups ($p<0.05$), while there was no difference in the allele distribution frequency between the two groups ($p>0.05$). The recessive model had differences between the two groups ($p<0.05$), while the dominant and additive model had no differences between the two groups ($p>0.05$).

CONCLUSIONS: HIE is correlated with maternal factors, fetal growth, uterine environment and labor process, and the rs2067853 polymorphism in AGT gene is associated with HIE.

Key Words:
Neonates, Hypoxic-ischemic encephalopathy, Factors, Angiotensinogen, Single nucleotide polymorphism.

Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) refers to the brain cell damage, organ dysfunction and metabolic disorders of blood glucose and electrolyte caused by various factors in the perinatal period, which is the main cause of neonatal death and neuronal developmental disorder, as well as a serious complication of neonatal asphyxia. In severe cases, irreversible brain damage can occur, resulting in permanent neurological dysfunction. Most previous studies argued that fetal distress is the core factor of HIE. With the deepening of the research, however, it has been found that HIE is also correlated with congenital genetic factors, and gene mutations may affect the occurrence and development of HIE[1-4]. The current study has also shown that the angiotensinogen (AGT) gene of the renin-angiotensin system is associated with the occurrence of cardio-cerebrovascular diseases[5,6]. However, there is a lack of relevant study on the correlation between the pathogenesis of neonatal HIE and AGT gene polymorphism. Therefore, this work was conducted to test the rs2067853 polymorphism in the AGT gene with TaqMan-MGB probe method by collecting the data of neonates with HIE from our department and explore the correlation between the AGT gene polymorphism and the pathogenesis of HIE, to provide theoretical support for the study on HIE genetic polymorphism.

Patients and Methods

Study Subjects

Neonates with HIE who were treated in the Pediatric Department of Zhengzhou University Affiliated Children's Hospital from January 2016 to January 2018 were selected. The diagnostic criteria for neonatal HIE in *Practical Neonatology* were applied to the diagnosis of HIE: (1) Neonatal hypoxia-ischemia

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is caused by perinatal asphyxia. (2) Abnormal neurological symptoms start at 1-2 hours after delivery, including disturbance of consciousness, alteration of muscle tone and primitive reflex abnormality. (3) Cerebral edema is confirmed by craniocerebral ultrasound, while brain atrophy is often confirmed by brain CT. (4) Parents of neonatal patients were informed and agreed. Exclusion criteria: (1) Neonates with purulent meningitis. (2) Neonates with asphyxia caused by other reasons. According to the criteria above, a total of 96 neonates with HIE were included in this study, including 63 male neonates and 33 female neonates, 69 term infants, 15 premature infants and 12 post-term infants. Meanwhile, a total of 123 non-asphyxia neonates treated in the same period in our department were selected as controls, including 82 male neonates and 41 female neonates, 85 term infants, 21 premature infants and 17 post-term infants. All subjects were unrelated neonates, and all their family members signed the informed consent. This study was approved by the Ethics Committee of Zhengzhou University Affiliated Children's Hospital.

Patients and Methods

Collection of General Clinical Data

Statistical analysis was performed on the data of neonates in both groups using questionnaires. The influencing factors included the advanced maternal age (>35 years old), low maternal age (<19 years old), pregnancy-induced hypertension or eclampsia, maternal renal insufficiency, maternal anemia, routine prenatal examination, natural childbirth, abnormal labor, amniotic fluid contamination, umbilical cord abnormality, placental abnormality and abnormal birth weight (2.50 -4.00 kg in normal cases). Questionnaires were completed and evaluated by two highly-trained and qualified doctors.

Deoxyribonucleic Acid (DNA) Extraction

A total of 1 mL of femoral venous blood was taken from neonates. The DNA was extracted with the Medium Scale Blood Genomic DNA Extraction Kit (Beijing Biotech Co., Ltd., Beijing, China) strictly according to the instructions of the kit. Moreover, the genotype of samples was detected and analyzed using the TaqMan®SNP Genotyping Assays kit (Thermo-Fisher, Waltham, MA, USA). The specific gene locus probes are shown in Table I.

Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM, Armonk, NY, USA). Enumeration data were compared between two groups *via* chi-square test. The likelihood-ratio χ^2 test was adopted to analyze whether the distribution of each genotype was consistent with the Hardy-Weinberg equilibrium. The R×C chi-square test was adopted for the comparison of the frequency of genotype and allele in each group. $p<0.05$ represented that the difference was statistically significant.

Results

Comparison of Basic Data

There were no differences in fetal gender and gestational age between the two groups ($p>0.05$) (Table II).

Comparison of Influencing Factors for Neonates

The frequency of advanced maternal age, low maternal age, maternal renal insufficiency, abnormal labor, amniotic fluid contamination and umbilical cord abnormality in the observation group was higher than that in the control group ($p<0.05$), while there was no difference between the two groups in the frequency of pregnancy-induced hypertension or eclampsia, maternal anemia, routine prenatal examination, natural childbirth, placental abnormality and birth weight abnormality ($p>0.05$) (Table III).

Table I. TaqMan®-MGB probe in rs2067853 gene locus in AGT gene.

SNP Reference	rs2067853
Assay ID	C_204369_20
Protein ID	NP_000020.1
SNP Type	Intron
Context Sequence	GTATACATCTGTTTGGCTGCTAAAT[A/G]AAAGATAAAATTTTTGGAGGCTTAT

Table II. Comparison of basic data between the two groups [n (%)].

Group	No.	Male/Female	Term infant/premature infant/post-term infant
Observation group	96	63 (65.63)/33 (34.37)	69 (71.88)/15 (15.62)/12 (12.50)
Control group	123	82 (66.67)/41 (33.33)	85 (69.11)/21 (17.07)/17 (13.82)
χ^2		0.026	0.199
p		0.872	0.905

Table III. Comparison of influencing factors for neonates between the two groups [n (%)].

Influencing factor	Observation group (n=96)	Control group (n=123)	χ^2	*p*
Advanced maternal age	27 (28.26)	18 (14.81)	5.007	0.025
Low maternal age	35 (36.96)	9 (7.41)	26.224	0.000
Pregnancy-induced hypertension or eclampsia	19 (19.57)	14 (11.11)	3.092	0.079
Maternal renal insufficiency	21 (21.74)	5 (3.70)	14.324	0.000
Maternal anemia	15 (15.22)	9 (7.41)	3.269	0.071
Routine prenatal examination	93 (96.74)	112 (90.74)	3.191	0.074
Natural childbirth	72 (75.00)	100 (81.30)	1.270	0.260
Placental abnormality	29 (30.43)	11 (9.26)	14.047	0.000
Amniotic fluid contamination	19 (19.57)	13 (10.19)	3.922	0.048
Umbilical cord abnormality	19 (19.57)	11 (9.26)	4.880	0.027
Placental abnormality	17 (17.39)	13 (10.19)	2.098	0.147
Birth weight abnormality	18 (18.48)	11 (9.26)	3.468	0.063

Table IV. Hardy-Weinberg equilibrium test of rs2067853 genotype in AGT gene between the two groups.

		AA		AG		GG			
Group	No.	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	χ^2	*p*
Observation group	96	5	2.19	19	24.62	72	69.190	5.00	0.08
Control group	123	1	3.09	37	32.82	85	87.09	2.00	0.37

Table V. Comparison of AGT gene rs2067853 genotype distribution between the two groups [n (%)].

		Genotypes				
Group	No.	AA	AG	GG	χ^2	*p*
Observation group	96	5 (5.21)	19 (19.79)	72 (75.00)	6.296	0.043
Control group	123	1 (0.81)	37 (30.08)	85 (69.11)		

Hardy-Weinberg Equilibrium Test

The likelihood-ratio χ^2 test was adopted to compare the actual frequency and theoretical frequency of the three genotypes between the observation group and the control group. The distribution of genotype frequency of rs2067853 in AGT gene in both groups accorded with the Hardy-Weinberg equilibrium law ($p>0.05$), and they were comparable (Table IV).

Comparison of Genotype Distribution Frequency

The frequency of AA, AG and GG genotypes distribution was 5.21%, 19.79% and 75.00%, respectively, in the observation group, and 0.81%, 30.08% and 69.11%, respectively, in the control group. There were differences in the genotype distribution frequency between the two groups ($p<0.05$) (Table V).

Table VI. Comparison of AGT gene rs2067853A/G allele distribution between the two groups [cases (%)].

Group	No.	Allele		χ^2	*p*
		A	G		
Observation group	96	29 (15.10)	163 (84.90)	0.046	0.830
Control group	123	39 (15.85)	207 (84.15)		

Table VII. Genetic model analysis of rs2067853 in AGT gene between the two groups [n (%)].

	Item	Observation group	Control group	χ^2	*p*
Recessive model	AA vs. AG+GG	5 (5.21)/91 (94.79)	1 (0.81)/122 (99.19)	3.909	0.048
Dominant model	AA+AG vs. GG	24 (25.00)/72 (75.00)	37 (30.89)/86 (69.11)	0.923	0.337
Additive model	AA vs. AG vs GG	5 (5.21)/19 (19.79)/72 (75.00)	1(0.81)/36 (29.27)/86 (69.92)	5.923	0.052

Comparison of Allele Distribution Frequency

The distribution frequency of A and G alleles was 15.10% and 84.90%, respectively, in the observation group, and 15.85% and 84.15%, respectively, in the control group. There were no differences in the allele distribution frequency between the two groups ($p>0.05$) (Table VI).

Genetic Model Analysis of rs2067853 in AGT Gene

According to the genetic model analysis, there were differences in the recessive model between the two groups ($p<0.05$), and there were no differences in the dominant model and additive model between the two groups ($p>0.05$), indicating that the recessive model is suitable for describing the genetic model of rs2067853 in the AGT gene in HIE (Table VII).

Discussion

The pathogenesis of neonatal HIE is closely correlated with perinatal asphyxia. Studies have found that maternal factors, fetal growth, uterine environment, labor process and so on may cause HIE[7], and the pathological changes of HIE include cerebral edema, neuronal necrosis, gliosis, neurological damage and energy metabolic disorder in brain cells. If HIE is not actively and effectively treated in time, it will cause neonatal brain edema for more than a week, and even irreversible neuronal necrosis in severe cases, producing a serious impact on the health of neonates[8,9]. In addition, due to parents' insufficient understanding of neonatal health care and insufficient emphasis on the prenatal examination, there has been an increasing incidence of HIE over recent years. Therefore, it is of vital importance to analyze and understand the related risk factors for the pathogenesis of HIE.

The result of this study showed that the frequency of advanced maternal age, low maternal age, maternal renal insufficiency, abnormal labor, amniotic fluid contamination and umbilical cord abnormality in the observation group was higher than that in the control group, which is consistent with the findings of other scholars[10-12], indicating to some extent that advanced maternal age or low maternal age and renal insufficiency may affect the structure and function of the birth canal, leading to HIE. The above results also suggest that abnormal labor, amniotic fluid contamination and umbilical cord abnormality may increase the incidence of HIE in a direct way. Abnormal labor includes prolonged labor and precipitate labor. In particular, the prolonged second stage of labor can lead to the reduced blood supply to the placenta after uterine contraction, causing ischemia and hypoxia to the fetus, ultimately leading to HIE. Amniotic fluid contamination is mainly reflected in fetal hypoxia, and it is exactly the critical reason for the occurrence of HIE[13,14]. The umbilical cord is a cord-like structure that connects the placenta and the embryo, which is a major channel for the fetus to obtain nutrients from the maternal body and remove metabolites. The umbilical cord abnormality of infants may lead to obstacles in providing fetal nutrients and transporting metabolites, resulting in the occurrence of HIE[15,16]. In summary, medical staff should master the risk

factors of HIE, enrich specialized theories and improve operational skills, educate pregnant women in the perinatal period, closely monitor the labor process and properly treat various complications in a timely manner in clinical work, to reduce the incidence of HIE.

Current research has demonstrated that the pathogenesis of HIE is not only correlated with maternal factors, fetal growth, uterine environment and labor process but also associated with congenital genetic factors. AGT is located in the 1q42-43 segment encoding AGT, while rs2067853 is located at the 3' end of the AGT gene. Mutations in the AGT gene are closely correlated with the occurrence of cardio-cerebrovascular diseases[17-20]. Lanz et al[21] speculated that the mutation of the AGT gene may increase the level of angiotensin II indirectly by increasing the level of AGT in plasma. Angiotensin II may give rise to the contraction, hyperplasia and hypertrophy and lipid deposition of arterioles vascular smooth muscle, and cause the hardening of small subcutaneous vessels, thus resulting in ischemic changes in the subcortical and deep white matter. Therefore, mutations in the AGT gene may lead to ischemic changes in the neonatal brain cortex and deep white matter.

In this study, the polymorphism of rs2067853 (A/G) in AGT was selected and the genotype frequency and allele frequency of the observation group and the control group were analyzed using the TaqMan-MGB probe method. The result showed that there was a difference in the frequency of genotype distribution of rs2067853 (A/G) in the AGT gene between the two groups, suggesting that the polymorphism of rs2067853 (A/G) in the AGT gene is correlated with the risk of HIE. Further analysis of the A and G alleles in the two groups revealed that there was no difference in the distribution of A and G alleles between the two groups ($p>0.05$). Finally, genetic model analysis of rs2067853 in the AGT gene revealed that there were differences in the recessive model between the two groups ($p<0.05$), suggesting that the recessive model is suitable for describing the genetic model of rs2067853 in AGT gene of HIE.

Conclusions

We observed that the polymorphism of rs2067853 in the AGT gene is significantly correlated with the risk of HIE, and the risk of HIE is increased by recessive GG homozygous mutation of the rs2067853 (A/G) in the AGT gene.

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Conflict of interest

The authors declare no conflicts of interest.

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