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# The effect of single nucleotide polymorphisms of STAT3 on epilepsy in children

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**Abstract. – OBJECTIVE: To investigate the effect of single nucleotide polymorphisms (SNPs) of signal transducer and activator of transcription 3 (STAT3) on epilepsy in children.**

**PATIENTS AND METHODS: A total of 169 children suffering from epilepsy admitted in No. 1 People's Hospital of Jining from July 2015 to December 2016 were enrolled as the research subjects. Immunohistochemistry and real time-PCR were used for analysis of the expression of STAT3 and p-STAT3 in epilepsy patients. The genotypes and alleles of rs1053005 and re744166 were analyzed through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Statistical analysis was conducted to explore the correlation between the polymorphism of STAT3 and the incidence of epilepsy in children, and the polymorphism of STAT3 in the drug-resistant and non-resistant patients was compared.**

**RESULTS: Both the STAT3 and p-STAT3 were over-expressed in epilepsy patients. The GG genotype of rs1053005 was significantly lower in epilepsy patients than that of health control, $p<0.05$. By contrast, no significant difference was found in genotypes of rs744166 between epilepsy and healthy children. When comparing the genotypes of drug-resistant patients and that of non-resistant patients, the distribution of rs1053005 genotypes in the two groups showed a significant difference, $p<0.05$. No statistical difference was observed in rs744166 genotypes.**

**CONCLUSIONS: STAT3 polymorphism was associated with the risk of epilepsy and drug resistance to epilepsy. This study may provide a better understanding of STAT3 in epilepsy patients and provide new targets for the treatment of epilepsy patients.**

*Key Words:*

STAT3, Single nucleotide, Polymorphism, Rs1053005, Re744166, Epilepsy.

## Introduction

Epilepsy, with more than 65 million people afflicted[1], is the second most common neurological disorder, after stroke[2]. Patients with epilepsy usually exhibit varying degrees of dysfunction such as motion, feeling, awareness, behavior, and autonomy. According to the patients' performance at the time of seizure, epilepsy can be classified into generalized seizures, partial seizures, and atypical seizures[3]. Today, although many drugs are applied to treat epilepsy, around the two-thirds of the patients are seizure-free under pharmacological treatment[4]. In addition, drug resistance is still an important issue in epilepsy treatment[5]. Therefore, it is necessary to further investigate the pathogenesis and therapy of epilepsy.

The mechanism of epilepsy is complex and unclear, but reactive astrogliosis is a common pathological change in the hippocampus of patients with epilepsy[6]. It is believed that the over-excitation of local neurons in the brain leads to epilepsy with brain dysfunction by recurrent and paroxysmal discharge[7]. Recently, the relationship between gene polymorphism and the development of epilepsy attracted scholars' attention. Studies have shown that polymorphism in many related genes is associated with the development of epilepsy, such as GABBR1[8], ALDH2[9], and TIMP4[10]. It has been reported that the signal transducer and activator of transcription 3 (STAT3) played a role in the epilepsy of a rat model[11]. However, few studies focused on the effect of STAT3 in epilepsy patients, as well as the polymorphism of STAT3 in epilepsy patients.

In this research, we aimed to explore the association between single nucleotide polymorphisms (SNPs) of STAT3 and epilepsy in children. This

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study might provide a new understanding of the role of STAT3 in epilepsy and new targets for the treatment of epilepsy.

## Patients and Methods

### Patients

The present work included a total of 169 children patients who were diagnosed with epilepsy in our hospital from January 2015 to July 2016. Meanwhile, a total of 169 healthy individuals who received a physical examination in the same period were selected as the control group. All patients were diagnosed through consensus by at least two experienced neurologists as epilepsy and classified according to the International League Against Epilepsy Classification (1989)[12]. Patients with the following characteristics were excluded from the study: 1) significant psychiatric comorbidity; 2) history of pseudoseizures; 3) without reliable seizure frequency record; 4) presence of progressive or degenerative neurological or systemic disorder; 5) alcohol or drug abuse.

Subjects in the patient group were divided into drug-resistant group (n=101) and drug-responsive group (n=68) according to the following definitions[13]: 1) drug-resistant: patients were treated with more than two established antiepileptic drugs at the maximum tolerated dose, but obtained less than 50% reduction of seizure frequency or no change in the year before the date of blood collection; 2) drug responsive: patients with no less than 50% reduction or seizure free in the year before the date of blood collection.

Demographic data and basic clinical characteristics such as age, gender, predominant EEG lateralization, and family history of epilepsy of all participants in the study were investigated through questionnaire and patients' medical records. The study was approved by the Ethics Committee of No. 1 People's Hospital of Jining. The signed written informed consents were obtained from all participants before the study.

### Immunohistochemistry of STAT3 and p-STAT3

Immunohistochemistry was carried out as described previously[11]. Briefly, the brain tissues were collected and fixed in 10% buffered formalin after resection, Samples were then sectioned and spread, followed by deparaffinized, hydrated and incubated in 0.3% $H_2O_2$ for 15 min. Then sections were heated at 95°C for 15 min, blocked and incubated in the primary human anti-STAT3 (ab68153, 1: 200, Abcam, Cambridge, MA, USA) or anti-p-STAT3 (ab76315, 1: 100, Abcam, Cambridge, MA, USA) antibody overnight at 4°C, followed by incubation with corresponding secondary antibody. Finally, sections were stained by 3,3'-diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO, USA) and counterstained with Harris hematoxylin.

### Genotype

For each participant, 5 mL of anticoagulation peripheral blood sample was collected and stored at -40°C before sample processing. DNA was extracted according to the manufacturer's introduction using the Flexigene DNA kit (Qiagen, Hilden, Germany). The genotypes of rs105300 and rs744166 were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using the Sequence Detection System ABI 7500 (Applied Biosystems, Foster City, CA, USA). The primers used were as follows: STAT3 rs105300, F 5'-CCG GAT TTG GCA ACT CAA AAC-3', R 5'-TTA TGT ACT GAA GAG TGT TGC TGG-3'; STAT3 rs744166, F 5'-CTG GAG TAC AAA CCC TGA ACC-3', R 5'-TCC TGT GGC ATT TGG TAT TCAG-3'. The amplified PCR products were processed with restriction enzyme overnight (rs1053005 by DdeI, rs744166 by AluI) and 10% of the subjects were randomly selected for repeat analysis.

### Statistical Analysis

Data were expressed as mean ± SD (standard deviation) and n (%) of study participants, respectively. Independent continuous variables were compared using the Student's *t*-test. The categorical data were compared using the Chi-square test or the Fisher exact test to determine the Hardy-Weinberg equilibrium. A comparison of Cross-products [Odds ratio (OR)] with a 95% confidence interval (95% CI) was also recorded. A *p*-value was less than 0.05, and it was considered to be statistically significant. All analyses were conducted using Statistical Product and Service Solutions 18.0 (SPSS Inc., PASW Statistics for Windows, Chicago, IL, USA).

## Results

### Patient Characteristics

169 patients and the same number of healthy individuals were included in the study, with all

**Table I.** Characteristics of all participants.

| Variables | Patients, n = 169 | Control, n = 169 |
|---|---|---|
| Age, years | 9.32 ± 2.13 (2-14) | 9.15 ± 2.33 (2-14) |
| Gender, male: female | 97:72 | 95:74 |
| Predominant EEG lateralization, n (%) | | |
| Left | 36 (21.3) | |
| Right | 52 (30.8) | |
| Bilateral | 81 (47.9) | |
| Family history of epilepsy, n (%) | 31 (18.3) | |
| Drug-resistant epilepsy patients, n (%) | 68 (40.2) | |
| Non-resistant epilepsy patients, n (%) | 101 (59.8) | |

the characteristics shown in Table I. The average age of patients and controls were 9.32 ± 2.1 (2-14y) and 9.15 ± 2.3 (2-14y), respectively, including 68 cases of drug-resistance and 101 cases of non-resistance in the patients' group. All the characteristics showed no significant difference between patients and controls, $p>0.05$.

### The Expression of STAT3 and p-STAT3 Was Up-Regulated in Epilepsy Patients

To investigate the role of STAT3 in epilepsy patients, the expression of STAT3 and p-STAT3 in brain tissues was determined using IHC analysis. As shown in Figure 1, the expression of STAT3 was significantly up-regulated in brain

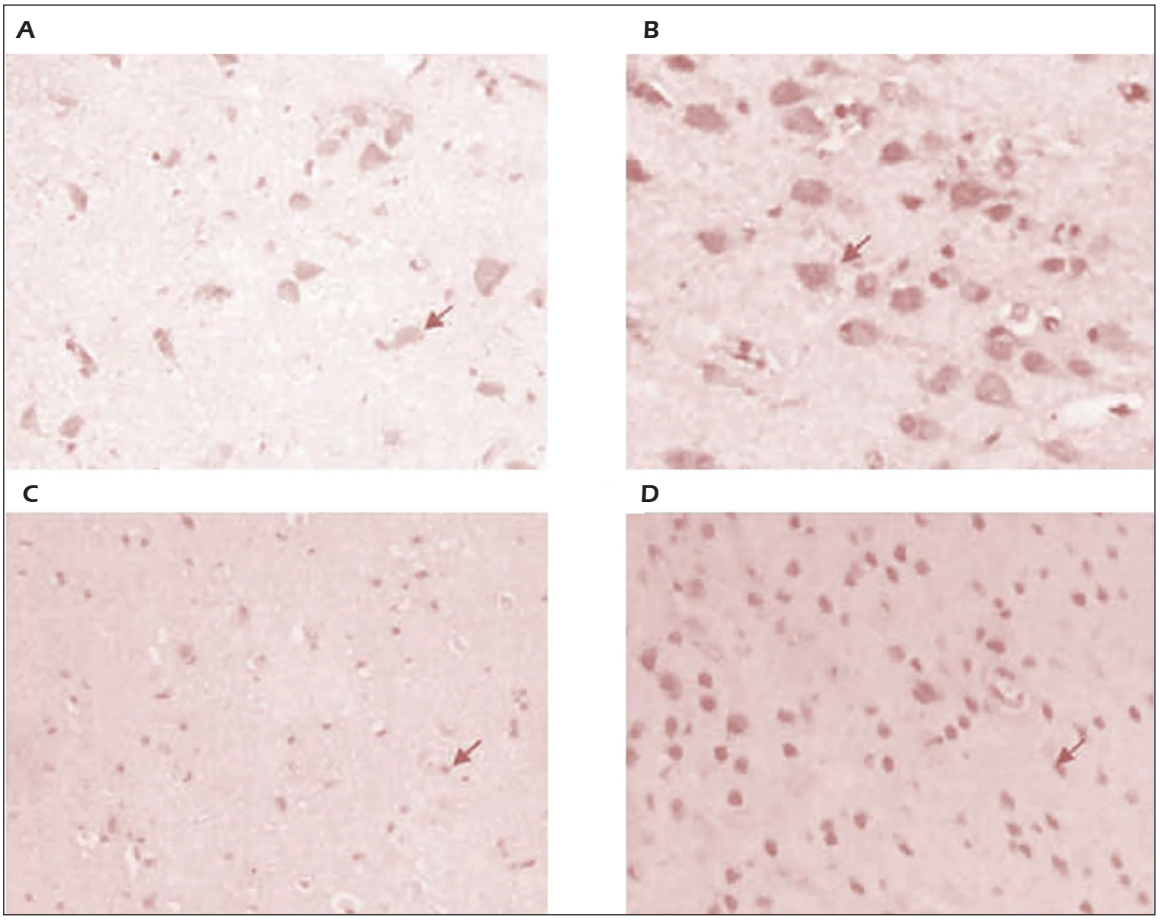


**Figure 1. A,** STAT3 immunofluorescence staining of the brain tissue of the healthy control; **B,** STAT3 immunofluorescence staining of the brain tissue of epilepsy patients; **C,** p-STAT3 immunofluorescence staining of the brain tissue of the healthy control; **D,** STAT3 immunofluorescence staining of the brain tissue of epilepsy patients. Magnification 200 ×.

tissues of the patients compared with the healthy control, suggesting both STAT3 and p-STAT3 were up-regulated in epilepsy patients.

### The Comparison of the Genotype of rs1053005 and rs744166 of STAT3 in Epilepsy Patients and Controls

As shown in Table II, the genotypes of rs1053005 and rs744166 in STAT3 were analyzed in all patients and controls. Results demonstrated that the distribution of STAT3 polymorphism genotypes of patients and controls was in Hardy-Weinberg equilibrium, $p>0.05$. Both the ratios of GG and AG+GG of STAT3 rs1053005 in epilepsy patients were significantly lower than that of the control, $p<0.05$, indicating that individuals with allele G might have lower risks for epilepsy. Meanwhile, the distribution of rs744166 polymorphism in STAT3 showed no significant difference between patients and the control group.

### Comparison of Genotypes in Drug-Responsive and Drug-Resistant Epilepsy Patients

To further investigate the effect of STAT3 polymorphism in epilepsy patients, a comparison of genotypes of rs1053305 and rs744166 in STATS were made between drug-responsive and drug-resistant patients. As displayed in Table III, the distribution of GG in rs1053305 of drug-responsive patients was significantly higher than that of drug-resistant epilepsy patients, $p<0.05$. However, the ratios of all the genotypes in rs744166 showed no difference in all the patients.

## Discussion

STAT3 is a kind of transcription factor which can regulate gene expression, increase genes that are important to cell survival, cell proliferation, cell-cycle progression and angiogenesis during normal development and epiletogenesis[14,15]. Recently, a growing number of studies have found that STAT3 plays an important role in astrocyte proliferation and differentiation after brain injury[16,17]. It was also reported that STAT3 was over-expressed in animal epilepsy model[18]. However, to the best of our knowledge, there is no study related to the effect of STAT3 gene polymorphism on epilepsy patients. This is the first study investigating the effect of single nucleotide polymorphisms of STAT3 on epilepsy. Firstly, we indicated that STAT3 and p-STAT3 were significantly over-expressed in the brain tissue of epilepsy patients compared with healthy controls. Xu et al[18] demonstrated that p-STAT3 was highly-expressed in the rat hippocampus during different phases of the epileptic process. Grabenstatter et al[11] used the pilocarpine model of acquired epilepsy and found that the inhibition of STAT3 showed a significant effect on status epilepticus and subsequent spontaneous seizures. All these results are consistent with our study.

Subsequently, we found that the ratio of GG (rs1053305 of STAT3) in healthy controls was higher than that of epilepsy patients, which meant that GG in rs1053305 of STAT3 might reduce the risk of epilepsy. Besides, no difference was found for rs744166 between the two groups. In the explo-

**Table II.** Comparison of genotypes for epilepsy patients and the control.

| Variable | Patient, n = 169, n (%) | Control, n = 169, n (%) | *p*-value | OR (95% CI) |
|---|---|---|---|---|
| **rs1053005** | | | | |
| Genotype | | | | |
| AA | 83 (49.1) | 58 (34.3) | | Ref=1 |
| AG | 70 (41.4) | 69 (40.8) | 0.272 | 1.188 (0.871-1.621) |
| GG | 16 (9.5) | 42 (24.9) | 0.002 | 1.446 (1.132-1.847) |
| AG+GG | 86 (50.9) | 111 (65.7) | 0.034 | 1.431 (1.022-2.005) |
| Allele | | | | |
| A | 236 (69.8) | 185 (54.7) | | Ref=1 |
| G | 102 (30.2) | 153 (45.3) | 0.028 | 1.276 (1.024-1.590) |
| **rs744166** | | | | |
| TT | 71 (42.0) | 75 (44.4) | | Ref=1 |
| TC | 73 (43.2) | 72 (42.6) | 0.820 | 0.966 (0.717-1.301) |
| CC | 25 (14.8) | 22 (13.0) | 0.671 | 0.956 (0.776-1.177) |
| TC+CC | 98 (58.0) | 94 (55.6) | 0.732 | 0.946 (0.688-1.300) |
| Allele | | | | |
| T | 215 (63.6) | 222 (65.7) | | Ref=1 |
| C | 123 (36.4) | 116 (34.3) | 0.756 | 0.968 (0.789-1.188) |

**Table III.** Distribution of different genotypes (rs1053305 and rs744166) of STAT3 in drug-responsive and drug-resistant epilepsy patients.

| Variable | Drug-responsive, n = 101, n (%) | Drug-resistant, n = 68, n (%) | *p*-value | OR (95% CI) |
|---|---|---|---|---|
| **rs1053005** | | | | |
| Genotype | | | | |
| AA | 45 (44.6) | 38 (55.9) | | Ref=1 |
| AG | 43 (42.6) | 27 (39.7) | 0.320 | 0.875 (0.671-1.141) |
| GG | 13 (12.9) | 3 (4.4) | 0.020 | 0.837 (0.716-0.978) |
| AG+GG | | | | |
| Allele | | | | |
| A | 133 (65.8) | 103 (75.7) | | Ref=1 |
| G | 69 (34.2) | 33 (24.3) | 0.124 | 0.869 (0.726-1.040) |
| **rs744166** | | | | |
| TT | 41 (40.6) | 30 (44.1) | | Ref=1 |
| TC | 42 (41.6) | 31 (45.6) | 0.976 | 1.005 (0.742-1.361) |
| CC | 18 (17.8) | 7 (10.3) | 0.157 | 0.858 (0.693-1.061) |
| TC+CC | 60 (59.4) | 38 (55.9) | 0.616 | 0.921 (0.666-1.273) |
| Allele | | | | |
| T | 124 (61.4) | 91 (66.9) | | Ref=1 |
| C | 78 (38.6) | 45 (33.1) | 0.417 | 0.918 (0.746-1.130) |

ration of the genotypic distribution of rs1053305 and rs744166 in STATS between drug-responsive and drug-resistant patients, we demonstrated that GG genotype of rs1053305 was lower expressed in drug-resistant patients, indicating that patients with rs1053305GG genotype also had a lower risk for the development of drug-resistant epilepsy. Xiao et al[19] investigated the association of single-nucleotide polymorphisms in the STAT3 gene with autoimmune thyroid disease in Chinese individuals and found that the polymorphisms of rs1053005 were significantly associated with the risk of autoimmune thyroid disease. There are also a number of studies revealing that the polymorphisms of STAT3 are associated with the development and drug response of cancers[20,21]. Herein, STAT3 polymorphisms are firstly reported to be related to the risk of epilepsy. Due to the insufficient number and the relatively simple composition of cases, the study still has some limitations. A deep explanation of the effect of STAT3 polymorphism on epilepsy patients needs to be further studied and confirmed.

## Conclusions

We found that STAT3 and p-STAT3 were associated with epilepsy, and the ratio of GG genotype in rs1053305 of epilepsy patients was significantly higher than that of healthy controls. The ratio of GG in rs1053305 in drug-resistant patients was lower than that of drug-responsive patients. This study firstly demonstrated the effect of STAT3 polymorphism on epilepsy. Further studies are required for a better understanding of the association of STAT3 polymorphism with epilepsy.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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