Observational Study

Medicine®

OPEN

The level of *GNE* and its relationship with behavioral phenotypes in children with autism spectrum disorder

Xiaolei Yang, PhD[a,b,c], Hongjie Li, MD[a], Jie Ge, PhD[a], Hong Chao, PhD[a], Gang Li, MD[a], Zhongguang Zhou, PhD[c], Jicheng Liu, PhD[b,*]

Abstract

Autism spectrum disorder (ASD) is a serious nervous system disease, and the cause is not known. Sialic acid (SA) is an indispensable nutrient for early brain development. In previous study, it was found that the SA level of ASD group was lower than that of control group. However, the reason for this has not well explained. A case-control study was conducted to understand the association between the SA synthase enzyme regulatory gene and ASD. The study sample included 65 ASD children and 64 healthy children. The levels of the *GNE* gene were measured, which encodes UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE), a key enzyme in SA biosynthesis. The symptom severity, intelligence development level, and behavioral performance of ASD children were estimated. There was a significant difference in the levels of *GNE* between the ASD and control groups ($t = 2.028$, $P = .045$). Moreover, the levels of *GNE* were negatively related to stereotypical behaviors according to the Autism Diagnostic Observation Schedule (ADOS) assessment ($r = -0.386$, $P = .039$). However, there is no the correlation between the levels of *GNE* and autistic severity. As evaluated through the Social Responsiveness Scale (SRS), the levels of *GNE* were negatively associated with autistic mannerisms scores, social cognition scores and SRS total scores in the children with ASD ($r = -0.314$, $P = .020$). These results indicate that the *GNE* gene may be associated with autism spectrum disorder, and it is also related to autistic behavioral performance, such as stereotypical behaviors, autistic mannerisms, and social cognition ability. Our data suggest that future studies to explore the causal relationship between *GNE* and the etiology of ASD may be needed.

Abbreviations: ABC = autism behavior checklist, ADI-R = autism diagnostic interview–revised, ADOS = autism diagnostic observation schedule, ASD = autism spectrum disorder, BTBR = BTBR T+Itpr3tf/J mouse, CARS = childhood autism rating scale, CK = creatine kinase, GNE = UDP-GlcNAc 2-epimerase/ManNAc kinase, NCAM = neural cell adhesion molecules, PPVT = peabody picture vocabulary test, SA = sialic acid, SRS = social responsiveness scale, VABS = vineland adaptive behavior scale.

Keywords: autism spectrum disorder, behavioral phenotypes, *GNE* gene

1. Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental conditions involving social deficits, impaired communication, and repetitive stereotypical behaviors.[1] The prevalence of ASD in children and adolescents is 1 in 59 individuals in the United States; although high rates have been reported, the pathogenesis of ASD is still unknown.[2] Moreover, patients with ASD often show phenotypic heterogeneity.[3] Since the discovery of ASD, a large number of scholars have carried out etiological studies of ASD, including studies of neuroanatomy, brain imaging, genomics, proteomics, heredity, environmental factors, and epigenetics.[4–7] So far, some scholars believe that ASD has a strong correlation with genetic factors.[8] Although a large number of genes have been reported to correlate with ASD, the main effect gene of ASD has not been found, which brings great challenges to the diagnosis and treatment of ASD.[9] However, there are fewer studies on the association between the expression of genes in the blood and their behavioral phenotypes in patients with ASD.

The *GNE* gene takes part in encoding UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE), which is a bifunctional enzyme that initiates and regulates the biosynthesis of sialic acid (SA).[10] SA is an essential nutrient for brain development and cognition and is also a necessary component of brain gangliosides.[11] Gangliosides are the most abundant sialoglycans expressed in nerve cells

Editor: Wen-Jun Tu.

This work was supported by Youth Doctor Fund Projects of Qiqihar Medical Uinversity (QMSI2019B-04) and the University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (UNPYSCT-2018036).

The authors declare no competing financial interests.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

[a] Department of Preventive Medicine, School of Public Health, [b] Postdoctoral Workstation, Research Institute of Medical and Pharmacy, Qiqihar Medical University, Qiqihar, [c] Postdoctoral Research Station, Heilongjiang University of Chinese Medicine, Harbin, China.

[*] Correspondence: Jicheng Liu, Postdoctoral Workstation, Research Institute of Medical and Pharmacy, Qiqihar Medical University, Qiqihar 161006, Heilongjiang, China (e-mail: qyybliu@126.com).

How to cite this article: Yang X, Li H, Ge J, Chao H, Li G, Zhou Z, Liu J. The level of GNE and its relationship with behavioral phenotypes in children with autism spectrum disorder. Medicine 2020;99:28(e21013).

Received: 20 March 2020 / Received in final form: 30 April 2020 / Accepted: 28 May 2020

http://dx.doi.org/10.1097/MD.0000000000021013

and may play an important role in axon-myelin interactions, axon stability, axon regeneration, and modulation of nerve cell excitability.[12] Ganglioside levels are increased in the cerebrospinal fluid from children with ASD.[13,14] Furthermore, SA could be synthesized into polysialic acid glycan, which associates with neural cell adhesion molecules (NCAM) to form PolySia-NCAM, which in turn impacts molecular interactions during synaptic plasticity and neural development.[15,16] The expression of PolySia-NCAM was decreased in the brain tissue of both valproic acid induced autistic model rats and BTBR T+Itpr3tf/J mouse (BTBR). An case-control study found that the levels of SA in the peripheral blood of children with ASD were lower than those of controls.[17] Additional, 1 study found that levels of salivary SA in children with ASD was different from healthy children, and it was related to autistic behaviors.[18] The above evidence suggests that the SA signal pathway may be associated with ASD. Therefore, more comprehensive studies are still required to confirm this hypothesis.

Based on the above findings, the abnormal synthesis of GNE may lead to dysfunction and the inability to synthesize enough SA. Therefore, we suspect that abnormalities in the expression of the *GNE* gene may be involved in the unusual regulation of GNE synthesis in patients with ASD. In addition, we want to explore whether the level of *GNE* is associated with behavioral phenotypes of children with ASD. Therefore, we conducted a case-control study to confirm the above hypotheses.

2. Methods

2.1. Patients

The case-control study contained 65 children with ASD (10 girls, 55 boys, age 4.45 ± 0.98) and 64 typically developing children (15 girls, 49 boys, age 4.43 ± 0.72). All children with ASD were diagnosed by a psychiatrist based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition and the combined results of the Autism Diagnostic Interview–Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS). The exclusion criteria were fragile X syndrome, attention deficit hyperactivity disorder, tic disorder, and mental retardation. Control children were recruited from those who attended kindergartens or elementary schools in Harbin and Qiqihar cities, agreed to mental and neurological examinations, and did not exhibit any developmental or nervous system diseases. This study was approved by the Ethical Committee of Qiqihar Medical University (No.201920) in the year of 2019. In addition, an informed written consent form for participation in the study was signed by the parents or legal guardians of all study subjects.

2.2. Evaluation of behavioral symptoms in ASD children

Based on clinical history and neuropsychiatric assessment, the children with ASD or their caregivers completed the Peabody Picture Vocabulary Test (PPVT), the Autism Behavior Checklist (ABC), the Childhood Autism Rating Scale (CARS), the Vineland Adaptive Behavior Scale (VABS), the Social Responsiveness Scale (SRS) and the neuropsychological development examination table for children aged 0-6 years old. The PPVT is used to examine receptive vocabulary ability and to assess intellectual development in children.[19] The ABC, which was completed by the parents, was developed to assess and screen for autism and to evaluate the severity of autism symptoms.[20] CARS is a commonly used tool for diagnosing autism. Scores ranging from 30 to 36 were categorized as mild to moderate autism, and scores ranging from 37 to 60 were considered severe autism.[21] The SRS has been validated, and has been shown to be reliable and have a good correlation with the gold-standard ADI-R.[22] The VABS is used to examine core autistic behaviors and focuses on communication and adaptive behaviors.[23]

2.3. Measurement of GNE levels

Blood samples were obtained between 8:30 and 11:00 in the morning, and RNA was immediately extracted from fresh blood. Total RNA was extracted from fresh blood of the children with ASD and healthy controls using the RNAprep pure Blood Kit (TIANGEN BIOTECH (BEIJING) CO., LTD). Clearance of DNA contamination in the RNA samples and cDNA synthesis was performed using the PrimeScript RT reagent Kit with gDNA Eraser according to the manufacturers instructions (TaKaRa Bio). Real-time PCR was subsequently performed using the ABI-7500 System employing the SYBR Select Master Mix (Applied Biosystems, Life-Technologies). The specific RT-PCR primers for *GNE* were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Primer sequences were:

GNE:	Forward	5′- CAATGATGGCAACTGTGCTG-3′
GNE:	Reverse	5′- AATTCCACCACCGATTCCTG-3′
HS-ACTB:	Forward	5′- CCTGGCACCCAGCACAAT-3′
HS-ACTB:	Reverse	5′- GGGCCGGACTCGTCATAC-3′

2.4. Statistical analysis

Data were prospectively collected and analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). For the descriptive data, we computed the means and standard deviations of demographic and outcome variables (independent t test and one-way ANOVA). We used the Chi-Squared test, Fisher exact test and the Kruskal-Wallis H test to determine differences in the distribution of categorical variables (PPVT, ABC, CARS scores) in different groups. The correlation between *GNE* levels and the behavioral phenotype of children with ASD was determined using Pearson and Spearman correlation analysis. For all analyses, statistical significance was set at a P value of .05.

3. Results

3.1. The levels of GNE in ASD and control groups

There was no age or gender difference between the ASD and the control groups ($P = .931$, .247, respectively). There was significant difference in the levels of *GNE* between the ASD and control groups ($t = 2.028$, $P = .045$) (Fig. 1). No statistically significant difference was found in different ages and genders between the children of ASD and control groups ($P > .05$).

3.2. The relationship between the levels of GNE and behavioral symptoms in ASD children

Through Spearman correlation analysis, it was found that *GNE* levels were negatively related to stereotypical behavior scores of ADOS scale ($r = -0.386$, $P = .039$, $n = 29$). It was shown that the lower the expression levels of *GNE* were, the higher the stereotypical behavior scores in the children with ASD who had

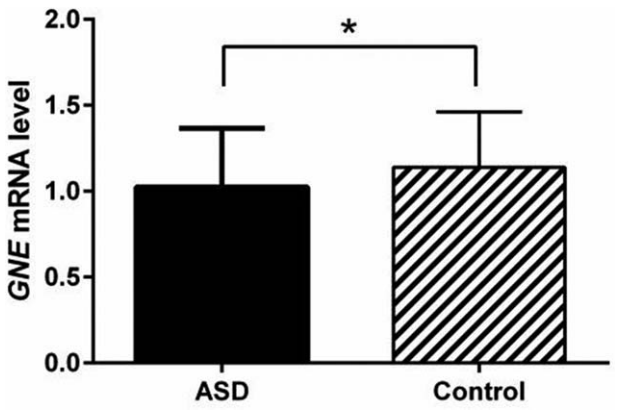

Figure 1. The *GNE* mRNA level in the ASD and control groups.

more serious stereotype behavior problems (Fig. 2). There was no significant difference in the *GNE* levels among different ASD severity levels or different intelligence development levels of children with ASD (Table 1). Within the ASD group, the levels of GNE were negatively correlated with the autistic mannerisms scores and social cognition scores of the SRS scale ($P < .05$). It indicated that the lower the level of *GNE* was, the higher the autistic behavior score, and the more serious the autistic behavior problem. However, *GNE* levels were not correlated with various field scores on the VABS scale (see Table 2).

4. Discussion

To the best of our knowledge, this is the first study on *GNE* levels in peripheral blood and their association with behavioral phenotypes in children with ASD. There was a decrease in the *GNE* levels of peripheral blood in ASD group compared with the healthy controls. Additionally, the *GNE* levels were negatively related to autistic mannerisms scores and social cognition scores of SRS scale, which was used to evaluate social behaviors. This meant that the lower levels of *GNE* in children with ASD were associated with serious autistic and social problems. Moreover, ASD children with the lower the levels of *GNE* may perform more serious stereotypical behavioral problem.

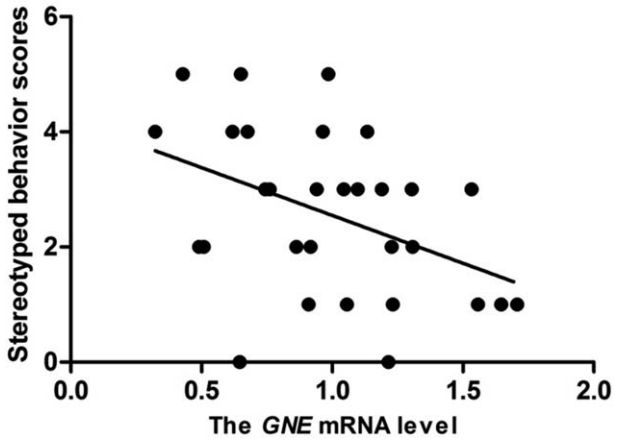

Figure 2. The correlation between *GNE* mRNA level and stereotypical behavior scores of ADOS scale.

Table 1

The relationship between the severity and intelligence development of autistic children and *GNE* mRNA levels.

Item	N	*GNE* (Mean±SD)	*F*	*P*
ABC scores				
≤53	27	1.09±0.37	1.698	.192
54-66	12	0.90±0.32		
≥67	24	0.97±0.28		
CARS scores				
<30	15	1.02±0.26	0.041	.960
30-36	44	0.99±0.36		
37-60	3	0.97±0.17		
PPVT scores				
<70	40	1.01±0.34	1.776	.179
70-85	13	0.99±0.20		
>85	6	1.27±0.45		

In this study, 2 ASD child did not complete the ABC scale, 3 ASD children did not complete the CARS scale, and 6 ASD children did not complete the PPVT test.
ABC = Autism Behavior Checklist, PPVT = Peabody Picture Vocabulary Test, CARS = Childhood Autism Rating Scale.

Sialic acid, a family of 9-carbon sugar acids, is an integral structural and functional component of the nervous system, particularly as precursors for the synthesis of the polysialic acid glycan, which modify the cell membrane-associated NCAM.[15] SA is synthesized in the cytoplasm and transported into the nucleus where it is activated with cytidine triphosphate to form the activated nucleotide donor of SA, cytidine monophosphate sialc acid.[24] It exerts negative feedback inhibition on GNE, the key bifunctional cytoplasmic enzyme that regulates the biosynthesis of SA, thus limiting excess production of free SA.[25] During childrens learning and cognition development, the surfaces of nerve cells need to form a large number of PolySia-NCAM complexes; so that the demand for SA also subsequently increases.[15] Therefore, both GNE and SA levels might affect the development of learning and cognitive function. However, a low level of learning and cognitive ability is common in children with ASD, so it is important to carry out a study to understand the correlation between the SA synthetase regulating gene (*GNE*) and ASD.[26,27]

Table 2

The relationship between *GNE* level and adaptive and social behavior in children with autism spectrum disorder.

Item	*r*	*P*
VABS total standard scores	0.094	.482
Communication	0.117	.382
Daily living skills	0.055	.681
Socialization	0.223	.093
Motor skills	0.066	.621
SRS total scores	−0.306	.023
Social awareness	−0.085	.538
Social cognition	−0.272	.044
Social communication	−0.243	.074
Social motivation	−0.199	.144
Autistic mannerisms	−0.314	.020

In this study, 7 ASD children did not complete VABS scale and 10 ASD children did not complete SRS scale.
VABS = Vineland Adaptive Behavior Scale, SRS = Social Responsiveness Scale.

GNE myopathy is caused by a mutation in the *GNE* gene, and the level of creatine kinase (CK) in these patients is increased.[28,29] Abnormal expression of CK and the creatine kinase brain-band isoenzyme was found in the peripheral blood of patients with ASD.[30,31] CK will affect the function of skeletal muscle, exercise ability, and behaviors. Patients with ASD are often characterized by low levels of gross motor ability and social behavior disorder. In addition, 2 studies reported that motor skills (e.g., stability, motor accuracy, and object manipulation) were negatively related to social function in children with ASD.[32,33] However, there is no report on whether the *GNE* gene is associated with motor ability, social behavior and other behaviors in patients with ASD. Interestingly, there was a difference in *GNE* gene expression between the children with ASD and control children in this study. The reasons for the low expression of the *GNE* gene in the ASD group still need more systematic study.

The main core symptoms of ASD are social impairment and stereotypical behavior.[1] However, in this study, we found that *GNE* levels were negatively correlated with autistic mannerisms, social cognition and stereotypical behaviors according to the SRS and ADOS scales. Autistic-like behaviors have a great impact on the education, life and health of ASD children. Improving the core symptoms, providing a reasonable intervention ASD treatment and ameliorating the quality of life for children with ASD may have profound significance. Moreover, this study could provide new evidence for the etiological study of ASD.

There are some limitations to this study. First, the level of UDP-GlcNAc 2-epimerase/ManNAc kinase in the children with ASD was not measured. Apart from this, an increased number of samples may be needed in a follow-up study. Second, as a nervous system disease, determining the levels of the *GNE* gene and GNE in the brain tissue of patients with ASD will provide more specific evidence to elucidate the relationship between *GNE* and ASD. In the follow-up study, the mutation of *GNE* in patients with ASD should be further evaluated. Third, this work was a case-control study; thus, there was a limitation in verifying the causal relationship between *GNE* and ASD. Therefore, an autistic mouse model will be used to further verify the exact association between *GNE* and ASD in subsequent studies.

Author contributions

Data curation: Xiaolei Yang and Jie Ge.
Evaluation of behavioral symptoms: Hongjie Li, Hong Chao and Gang Li.
Measurement of *GNE* levels: Xiaolei Yang and Jie Ge.
Project administration: Xiaolei Yang.
Writing – original draft: Xiaolei Yang.
Writing – review & editing: Zhongguang Zhou, Jicheng Liu.

References

[1] Sharon G, Cruz NJ, Kang DW, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. Cell 2019;177:1600–18. e1617.
[2] Baio J, Wiggins L, Christensen DL, et al. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2014. Morbidity and mortality weekly report. Surveillance Summaries (Washington, D C : 2002) 2018;67:1–23.
[3] Tordjman S, Cohen D, Coulon N, et al. Reframing autism as a behavioral syndrome and not a specific mental disorder: implications of genetic and phenotypic heterogeneity. Neurosci Biobehav Rev 2017;80:210.
[4] Piven J, Elison JT, Zylka MJ. Toward a conceptual framework for early brain and behavior development in autism. Mol Psychiatry 2017;22: 1385–94.
[5] Shen L, Feng C, Zhang K, et al. Proteomics Study of Peripheral Blood Mononuclear Cells (PBMCs) in autistic children. Front Cell Neurosci 2019;13:105.
[6] Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 2012;14:281–92.
[7] Ecker C. The neuroanatomy of autism spectrum disorder: an overview of structural neuroimaging findings and their translatability to the clinical setting. Autism 2017;21:18–28.
[8] Fakhoury M. Autistic spectrum disorders: a review of clinical features, theories and diagnosis. Int J Dev Neurosci 2015;43:70–7.
[9] Pain O., Pocklington A.J., Holmans P.A., et al. Novel insight into the etiology of autism spectrum disorder gained by integrating expression data with genome-wide association statistics. Biol Psychiatry 2019;86: 265–273.
[10] Carrillo N, Malicdan MC, Huizing M. GNE myopathy: etiology, diagnosis, and therapeutic challenges. Neurotherapeutics 2018;15: 900–14.
[11] Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. Physiol Rev 2014;94:461–518.
[12] Schnaar RL. Gangliosides of the vertebrate nervous system. J Mol Biol 2016;428:3325–36.
[13] Lekman A, Skjeldal O, Sponheim E, et al. Gangliosides in children with autism. Acta Paediatrica (Oslo, Norway: 1992) 1995;84:787–90.
[14] Nordin V, Lekman A, Johansson M, et al. Gangliosides in cerebrospinal fluid in children with autism spectrum disorders. Dev Med Child Neurol 1998;40:587–94.
[15] Wang B. Molecular mechanism underlying sialic acid as an essential nutrient for brain development and cognition. Adv Nutr 2012;3: 465s–72s.
[16] Cremer H, Chazal G, Lledo PM, et al. PSA-NCAM: an important regulator of hippocampal plasticity. Int J Dev Neurosci 2000;18:213–20.
[17] Yang X, Liang S, Wang L, et al. Sialic acid and anti-ganglioside antibody levels in children with autism spectrum disorders. Brain Res 2018;1678: 273–7.
[18] Demirci E, Guler Y, Ozmen S, et al. Levels of salivary sialic acid in children with autism spectrum disorder; could it be related to stereotypes and hyperactivity? Clin Psychopharmacol Neurosci 2019;17:415–22.
[19] Melamed IR, Heffron M, Testori A, et al. A pilot study of high-dose intravenous immunoglobulin 5% for autism: impact on autism spectrum and markers of neuroinflammation. Autism Res 2018;11:421–33.
[20] Krug DA, Arick J, Almond P. Behavior checklist for identifying severely handicapped individuals with high levels of autistic behavior. J Child Psychol Psychiatry 1980;21:221–9.
[21] Meng WD, Sun SJ, Yang J, et al. Elevated Serum Brain-Derived Neurotrophic Factor (BDNF) but not BDNF gene Val66Met polymorphism is associated with autism spectrum disorders. Mol Neurobiol 2017;54:1167–72.
[22] Constantino J, Davis S, Todd R, et al. Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the autism diagnostic interview-revised. J Autism Dev Disord 2003;33:427–33.
[23] Mandic-Maravic V, Pejovic-Milovancevic M, Mitkovic-Voncina M, et al. Sex differences in autism spectrum disorders: does sex moderate the pathway from clinical symptoms to adaptive behavior? Sci Rep 2015;5:10418.
[24] Sellmeier M, Weinhold B, Munster-Kuhnel A. CMP-sialic acid synthetase: the point of constriction in the sialylation pathway. Top Curr Chem 2015;366:139–67.
[25] Bork K, Reutter W, Gerardy-Schahn R, et al. The intracellular concentration of sialic acid regulates the polysialylation of the neural cell adhesion molecule. FEBS Lett 2005;579:5079–83.
[26] Fernandez M, Mollinedo-Gajate I, Penagarikano O. Neural circuits for social cognition: implications for autism. Neuroscience 2018;370:148–62.
[27] Aldaqre I, Paulus M, Sodian B. Referential gaze and word learning in adults with autism. Autism 2015;19:944–55.
[28] Diniz G, Secil Y, Ceylaner S, et al. GNE myopathy in Turkish sisters with a novel homozygous mutation. Case Rep Neurol Med 2016;2016: 8647645.
[29] Sim JE, Park HJ, Shin HY, et al. Clinical characteristics and molecular genetic analysis of Korean patients with GNE myopathy. Yonsei Med J 2013;54:578–82.

[30] Al-Mosalem OA, El-Ansary A, Attas O, et al. Metabolic biomarkers related to energy metabolism in Saudi autistic children. Clin Biochem 2009;42:949–57.
[31] Lv MN, Zhang H, Shu Y, et al. The neonatal levels of TSB, NSE and CK-BB in autism spectrum disorder from Southern China. Transl Neurosci 2016;7:6–11.
[32] Holloway JM, Long TM, Biasini F. Relationships between gross motor skills and social function in young boys with autism spectrum disorder. Pediatr Phys Ther 2018;30:184–90.
[33] Pusponegoro HD, Efar P, Soedjatmiko , et al. Gross motor profile and its association with socialization skills in children with autism spectrum disorders. Pediatr Neonatol 2016;57:501–7.