

A Twins Case of Lissencephaly With GPR56 Compound Heterozygous Mutations and Literatures Review

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Research

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Abstract

Background: Lissencephaly (LIS) is a malformation of cortical development characterized by developmental delay and seizure in combination with wide gyrus, superficial sulcus, and thickened cortex. Up to date, 20 genes have been implicated in LIS. However, *GPR56*-related LIS has never been reported, which was considered one causative gene for bilateral frontoparietal polymicrogyria (BFPP).

Methods: Genetic testing of the proband was performed by whole exome sequencing and mainly analyzed 662 genes related to brain hypoplasia, white matter abnormalities, and hypothalamic-pituitary axis abnormalities such as AAAS, AARS2, ABAT, ABCC8, etc.. And the candidate mutations were further confirmed by polymerase chain reaction (PCR) and Sanger sequencing. Though we did genetic testing on the twin sisters and their parents, we could only obtain the clinical data of the older sister.

Results: We reported a case of LIS twins, from a nonconsanguineous family, both carried the novel compound heterozygous *GPR56* mutations, p.F76fs and p.H607fs. The older sister manifested lissencephaly, seizures, and flathead deformity and the younger sister had lissencephaly. We summarized their clinical characteristics and reviewed all the literatures of LIS and *GPR56* to further clarify the correlation between genotype and phenotype.

Conclusion: The LIS twins with *GPR56* mutations were the first reported. By reviewing the clinical manifestations of LIS and *GPR56* mutations, we validated the association between them, and broaden the clinical manifestations of *GPR56* related phenotypes, indicating the importance of *GPR56* screening in LIS patients.

1. Introduction

Lissencephaly (LIS) is a group of abnormal cerebral cortical dysplasia caused by the wrong migration of neurons. It can be diagnosed clinically by neuroimaging, presenting with thickening of the cerebral cortex, widening of the gyri, and the disappearance or shallowness of the sulcus. In severe cases, it can be agyria, which is manifested as the complete disappearance of the sulci and gyri, showing smooth surface of the brain^[1]. Both agyria and pachygyria belong to LIS. According to neuroimaging, LIS can be divided into 6 grades, ranging from severe agyria (grade 1) to mild subcortical band heterotopias (grade 6), and the severity of nerve damage is widely related to the degree of LIS and cortical thickening, and the mortality rate of severe LIS is high^[2]. In the early stage, patients often exhibit developmental delay and hypotonia, followed by seizures, and eventually have a severe intellectual disability. But it can be normal in the neonatal period. The main problems encountered in the neonatal are persistent feeding problems and many different types of epilepsy, which are usually difficult to cure^[3]. Rare individuals with mild lissencephaly and normal intelligence have been reported^[4]. Up to now, 20 genes have been implicated in LIS, many of which are microtubule genes^{[5][6]}.

GPR56(OMIM#606854, NM_0001145773)encodes an orphan G protein-coupled receptor which is widely expressed in the nervous system and is essential for the normal development of cerebral cortex and cerebellar morphology^[7, 8, 9]. At present, the mutations of *GPR56* have been confirmed to be related to bilateral frontoparietal polymicrogyria (BFPP)^[10].

In this study, we reported a case of LIS twins, coming from nonconsanguineous family, both carried a novel compound heterozygous *GPR56* mutation(Fig. 1). To our knowledge, they were the first *GRP56*-related LIS patients. The proband's clinical features were demonstrated retrospectively.

2. Methods

The probands were collected in the neurology department in Children's Hospital of Nanjing Medical University(Nanjing, PRC). The clinical materials were investigated in proband.

Genomic DNA was extracted from peripheral blood of the twins and their parents using the blood genomic DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen). Genetic testing of the proband was performed by high-throughput sequencing of the whole exome. The candidate mutations were further performed by PCR and Sanger sequencing. These mutations were also performed in the parents and her twin sister to further confirm.

Written informed consents were obtained from the patients. This study was approved by the ethics committee of Children's Hospital of Nanjing Medical University.

3. Results

3.1 Results of genetic testing

In the genetic testing, the mean depth of whole exon sequencing was 105 and the coverage was 99.73%. The percentage of the mean depth > 20X was 99.37%. According to the selection criteria for mutation sites: 1) The frequency of the normal population of dominant inheritance is less than 0.0002; the frequency of the normal population of recessive inheritance is less than 0.05; 2) remove synonymous mutations and regulatory region mutation sites. We found suspected pathogenic mutations (compound heterozygous) related to the subject's clinical symptoms): *GPR56* c.228delC (p.F76fs) and c.1820_1821delAT (p.H607fs), two mutations could cause the frame shift mutations, resulting in abnormal protein function.

Both mutation sites have been confirmed in the proband, their parents ,and twin sister by Sanger sequencing. The unaffected father carried a heterozygous c.1820_1821delAT mutation and the unaffected mother carried a heterozygous c.228delC mutation. The twin sister carried both mutations as the proband(Fig. 2).

Both mutation sites have not been reported in the Human Gene Mutation Database(HGMD, Professional edition). According to The American College of Medical Genetics and Genomics(ACMG) guideline rating, both variants were confirmed as PVS1 + PM2, which were suspected pathogenic mutations. And both variants were predicted to be disease causing by mutationtaster (mutationtaster.org) since the frame shift mutation took place in F76 and H607 which might cause nonfunctional protein product or splice site changes.

3.2 Clinical characteristics

The proband had a flat head deformity and manifested as developmental delay and suddenly generalized tonic-clonic seizure at five months without any causes. The pregnancy and delivery of proband were uneventful. The neurological examination, electroencephalogram(EEG), and laboratory findings (full blood count, liver, kidney, and thyroid function tests, creatine kinase, uric acid, metabolic study, and chromosome karyotyping) were normal. Brain Magnetic Resonance Image(MRI)revealed the brain structure was simple with widened and thickened gyrus and shallow sulcus. The white matter of the brain was significantly reduced. The patchy long T1 and long T2 signals could be seen around the ventricle which were expanded and the extracerebral space was widened (Fig. 3).

3.3 Literatures review

All thirty-three families including 58 patients carrying *GPR56* mutations reported till recently were reviewed(Table 1). According to the pedigree analysis, all the pedigrees showed a pattern of autosomal recessive inheritance. One case carried a compound heterozygous mutation and the others carried homozygous mutations. In these patients with available clinical data, developmental delay and epilepsy were the predominant symptoms. The brain MRI showed BFPP and white matter abnormalities, which often involving the brainstem and cerebellum, with or without developmental malformations of the corpus callosum.

4. Discussion

GPR56 spans 45 kb and consists of 14 exons, encoding an orphan G protein-coupled receptor(GPCR) which was constituted by 693 amino acids^[7, 11]. The G protein-coupled receptor belongs to the adhesion G protein-coupled receptor family, which has an N- and a C-terminal fragmental and a G Protein-Coupled Receptor proteolytic site^[12]. In central nervous system, GPR56 plays an important role in the normal development of the cerebral cortex and cerebellar morphogenesis^[8]. In the peripheral nervous system, CPR56 can regulate the formation and maintenance of myelin sheath^[13]. Therefore, the normal expression of the *GPR56* is essential for the function of nervous system. We have already known that the mutations of *GPR56* were related to BFPP. The patient's clinical manifestations were overall developmental delay, seizures, and MRI shows symmetrical polygyria (the frontal parietal area is the most serious part), ventricular enlargement, bilateral white matter changes, and 28 pathogenic *GPR56* mutations related to the BFPP phenotype have been reported^[11, 14]. All the individuals inherited in an autosomal recessive mode and almost all missense mutations showed similar clinical symptoms,

indicating that the similar phenotype might be caused by the same mechanism, but the mechanism was not clear, which might involve GPR56 trafficking and surface reduction of receptor expression^[15, 16, 17]. Knocking out the *GPR56* did not affect the migration of neural progenitor cells, while overexpressing *GPR56* could inhibit the migration of neural progenitor cells. This mechanism might be through the reorganization of cerebral cortex actin to change cell morphology to regulate neural progenitor cell behavior^[8]. We know that premature stop of neuronal migration could cause LIS, which might explain the mechanism of *GPR56* mutations causing the LIS to some extent.

The development of brain is a delicate and complex physiological process, and the proper migration of neurons is one of the most critical steps. LIS is the brain dysplasia caused by the premature stop of neuron migration. The typical lissencephaly (type I LIS) is characterized by the thickened cerebral cortex (10–20 mm, normal 4 mm), and there are no other forms of brain developmental malformations, such as severe congenital microcephaly, corpus callosum hypoplasia, or cerebellar hypoplasia^[2]. And it can be observed under the microscope that the cerebral cortex is divided into 4 thick and dysplastic layers: molecular layer, superficial cellular layer, cell spare layer, and deeper cellular layer, while the normal cerebral cortex has 6 layers^[1]. Up to now, 20 genes have been considered to be related to LIS, many of which are microtubule genes^{[5][6]}. In a cohort study of 811 patients with LIS, the overall mutation frequency of the entire cohort was 81%, of which *LIS1* accounted for 40%, followed by *DCX* (23%), *TUBA1A* (5%), and *DYNC1H1* (3%). Other genes account for 1% or less, and 19% of patients still have not found the cause, which indicates that other additional genes need to be discovered^[6]. In the past, there have been no reports about the *GPR56*-related LIS. Therefore, the relationship between LIS and *GPR56* still needs further research.

There is no specific treatment method for LIS, mainly symptomatic treatment such as anti-epileptic treatment and rehabilitation training. Studies in animal models have shown that it might be possible to restart neuronal migration by re-expressing the missing genes after birth. Even if the degree of cortical deformity was partially improved, it could also be significantly beneficial by controlling seizures and reducing the clinical severity^[2]. Therefore, with all the advances in genetic testing and medical technology, the diagnosis and treatment of LIS will continue to improve and progress.

5. Conclusion

In this case, the compound mutations of the *GPR56* in the LIS twin sisters are both novel mutations, which have not been reported in the HGMD professional database. This enriches the mutation database of *GPR56* and also broadened the clinical manifestation, increasing our understanding of *GPR56*. All these observations indicated that genetic testing is necessary when patients suffering from LIS symptoms.

6. Abbreviations

Brain Magnetic Resonance Image (MRI), bilateral frontoparietal polymicrogyria (BFPP), electroencephalogram (EEG), G Protein-Coupled Receptor (GPCR), Human Gene Mutation Database (HGMD), Lissencephaly (LIS), polymerase chain reaction (PCR), The American College of Medical Genetics and Genomics (ACMG)

7. Declarations

7.1 Ethics approval and consent to participate

This study was approved by the ethics committee of Children's Hospital of Nanjing Medical University. Written informed consents for participation were obtained from their parents.

7.2 Consent for publication

Written informed consents for publication were obtained from their parents.

7.3 Availability of data and material

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

7.4 Funding

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7.5 Competing Interests

The authors declare that they have no competing interests in this article.

7.6 Authors' contributions

Wenxin Lin performed the data analysis and drafted the manuscript. Yingying Chai conducted the molecular genetic studies and drafted the manuscript. Xia Zhang participated in the design of the study. Tingting Huang participated in the design of the study. Guo Zheng participated in the design of the study. Gang Zhang participated in the design of the study. Fang Peng participated in the design of the study. Yanjun Huang conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

7.6 Acknowledgements

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Conflict of interest

The authors report no conflicts of interest in this article.

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Table

Due to technical limitations, table 1 docx is only available as a download in the Supplemental Files section.

Figures

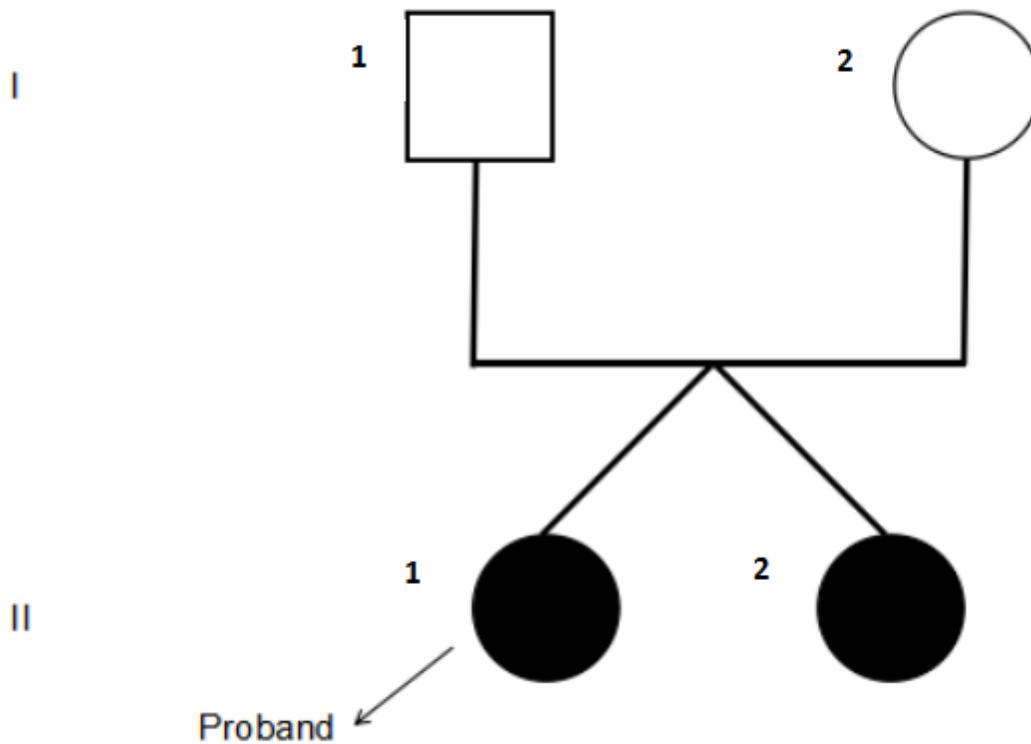


Figure 1

Pedigrees of family with GPR56 mutations. Arrow: proband; square: male; circle: female; solid symbol: affected.

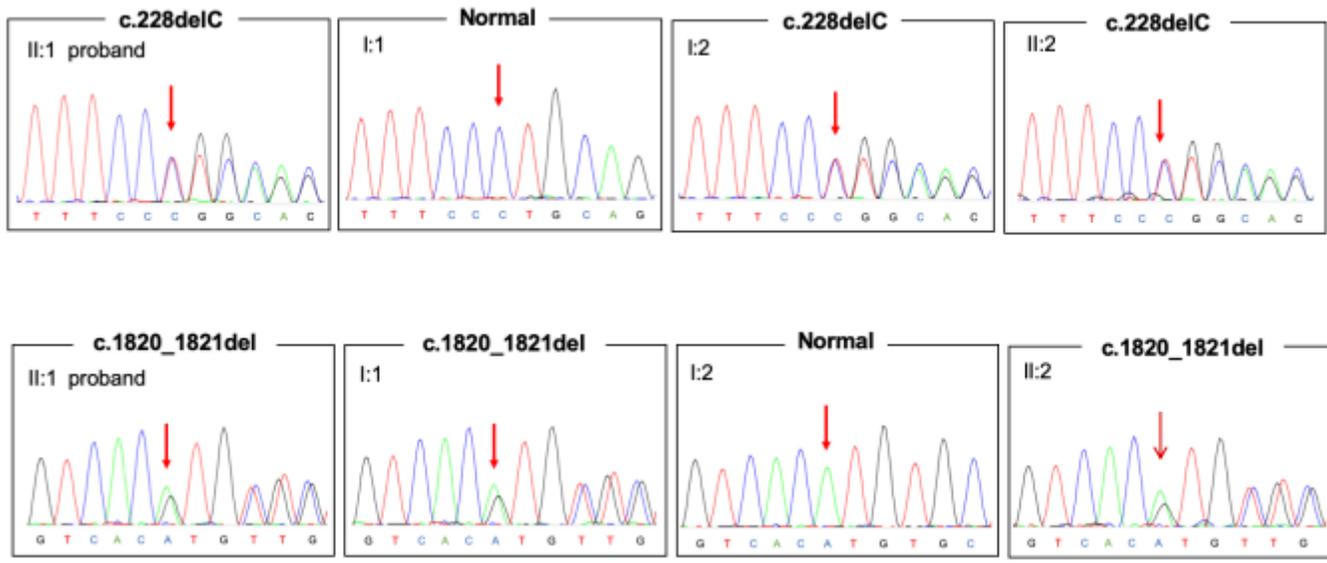


Figure 2

Sanger sequencing of proband and her family members.

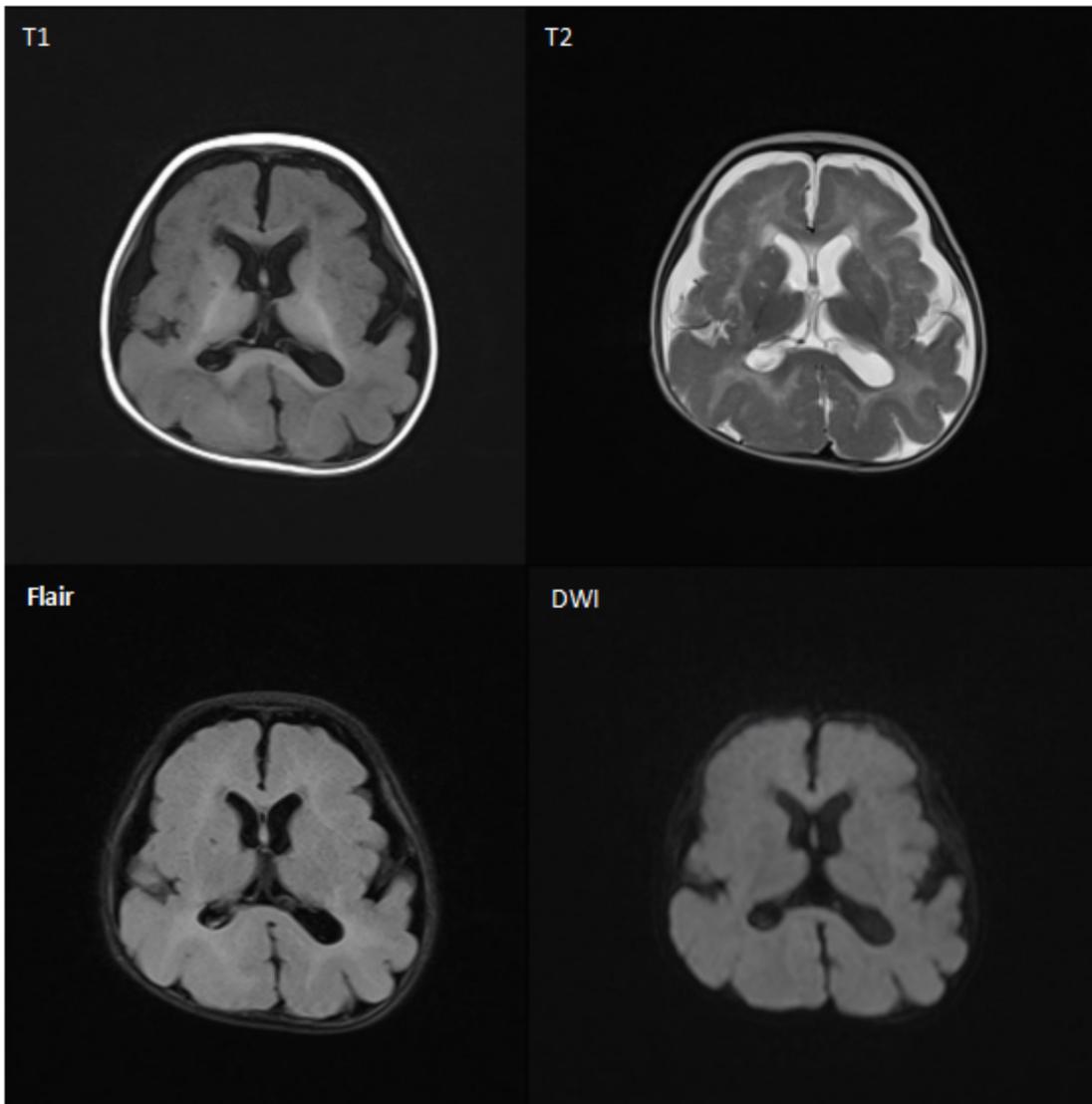


Figure 3

The brain MRI of proband revealed the brain structure was simple with widened and thickened gyrus and shallow sulcus.

Supplementary Files

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