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Greig cephalopolysyndactyly syndrome caused by novel GLI3 mutation: a family report

Ying Tang[1], Juan Ge[1], Tang Li[2]

[1]Department of Endocrinology and Metabolic Diseases, Qingdao University, Qingdao, Shandong 266000, China

[2]Department of Endocrinology and Metabolic Diseases, Qingdao Women and Children's Hospital, Qingdao, Shandong 266034, China

Abstract: **To investigate the clinical feature and gene mutaiton of Greig cephalopolysyndactyly syndrome (GCPS). The clinical data and the whole exome sequencing results of two patients with GCPS in the same family were retrospectively analyzed. The related literatures were reviewed. The propositus was a 7-month-old girl who manifested a prominent forehead and widely spaced eyes, but have no evident growth developmental retardation. Her mother, 25-year-old, manifested a prominent forehead, macrocephaly, hypertelorism, broad thumbs and halluces in both hands and feet, and scoliosis. A novel missense mutation in GLI3 gene was found by whole exon sequencing of the proband, which caused the 478th amino acid of the GLI3 gene encoded protein which changed from tyrosine to cysteine acid. The GLI3 mutation was identified in his mother by Sanger sequencing. Her father GLI3 gene was normal. The same missense mutation was not retrieved in the HGMD. Reports of the mutation were not found in the previous research. It may be a new mutation. The GCPS is a pleiotropic, multiple congenital anomaly syndrome caused by GLI3 gene mutation or deletion. More than 70 GLI3 gene mutation sites with GCPS had been reported and the most common type was frame shift mutations. Awareness by clinicians of the array of phenotypes manifest in GCPS and gene detection will aid in clinical diagnosis.**

Keywords: Greig cephalopolysyndactyly syndrome; GLI3 gene; Gene mutation

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*Corresponding Author: Tang Li, drlitang@hotmail.com

1. Introduction

Greig cephalopolysyndactyly syndrome (GCPS) was first reported in 1926 by Edinburgh surgeon David Middleton Greig[1], who described a mother and daughter with craniofacial abnormalities and polysyndactyly. GCPS is a rarely multi-directional congenital anomaly syndrome which was caused by mutations in the GLI3 gene. GLI3 gene inherited in an autosomal dominant pattern with very high penetrance in most of cases. The incidence is estimated to be within the range of 1–9/1,000,000[1]. It is known that GCPS is caused by mutation of the transcription factor GLI3 gene (7p13), which results in functional haploinsufficiency of this gene[2]. The current study found that the clinical phenotype of GCPS is non-specific, with extensive variability and complexity. The phenotypes of different GCPS individuals are significantly different, and the phenotypes of affected relatives in the same family are also different[3]. Firstly analyze the clinical characteristics and gene mutation characteristics of the family in GCPS with novel GLI3 mutation, then summarize the significant information by reviewing the relevant literature. Thereby we can raise the clinician's recognition of GCPS and improve the early diagnosis rate.

2. Case presentation

The proband who was a 5-month-old girl visited Qingdao Women and Children's Hospital for prominent forehead in December 2018. The proband is the first child of the parents. She was delivered by cesarean after a full term pregnancy. There was no obvious abnormality at her birth. Her mother denied a pregnancy history of viral infection, hazardous substances and radiation exposure. Her parents were not intermarriage and deny a similar genetic history in the family. The proband was breastfeeding after birth. She could lift her neck at 3-month-old, smile at 4-month-old and turn over at 5-month-old. On medical examination at 5-month-old, her height 61 cm (3th centile) and her OFC was 42 cm (50th centile). She had a broad forehead with frontal bossing and widely spaced eyes (Figure 1A). There was no obvious abnormality in her heart and lung by medical examination. Her vertebral column and muscular tension of the limbs was normal. The proband's mother, 25 years old, has a similar craniofacial abnormality, presenting macrocephaly with frontal bossing and hypertelorism (Figure 1B). The proband's mother complained that her fingers and toes were widened (Figure 1C) and her vertebral column was bent when she was 3 years old. The x-ray plain films of the proband mother's hands showed the second and third phalanx of the left hand. The proximal phalanx of the right hand 2-4th phalanges was irregular and enlarged, and proximal interphalangeal joint of the bilateral third phalanx was slightly deflected to the radius side. The 5th metacarpal of the right hand is short (Figure 1D, E).

The mother's vertebral column x-ray plain films showed the thoracolumbar spine is curved in an "S" shape, which centered on T8 and L1, and the intervertebral space of each vertebrae varies from wide to narrow (Figure 1F). We performed a whole exome sequencing and Sanger sequencing on the proband and her parents with informed consent of the proband's parents for purpose of accurate diagnosis. The results showed that the proband has a missense mutation in GLI3 gene: c.1433A>G (Figure 2), p.Y478C, which caused the 478th amino acid of the GLI3 gene protein encoded which changed from tyrosine to cysteine acid. According to Sanger sequencing, the GLI3 gene mutation in the child was derived from her mother, and her father had no variation at this site. We did not find any report about this new mutation site from HGMD database and literature search. The mutation was presumed to be a new mutation. The new mutation was predicted to be pernicious by the bioinformatics protein function prediction software SIFT, PolyPhen 2, and REVEL. Combining with the clinical manifestations and genetic test results of the proband and her mother, we considered the diagnosis was GCPS.

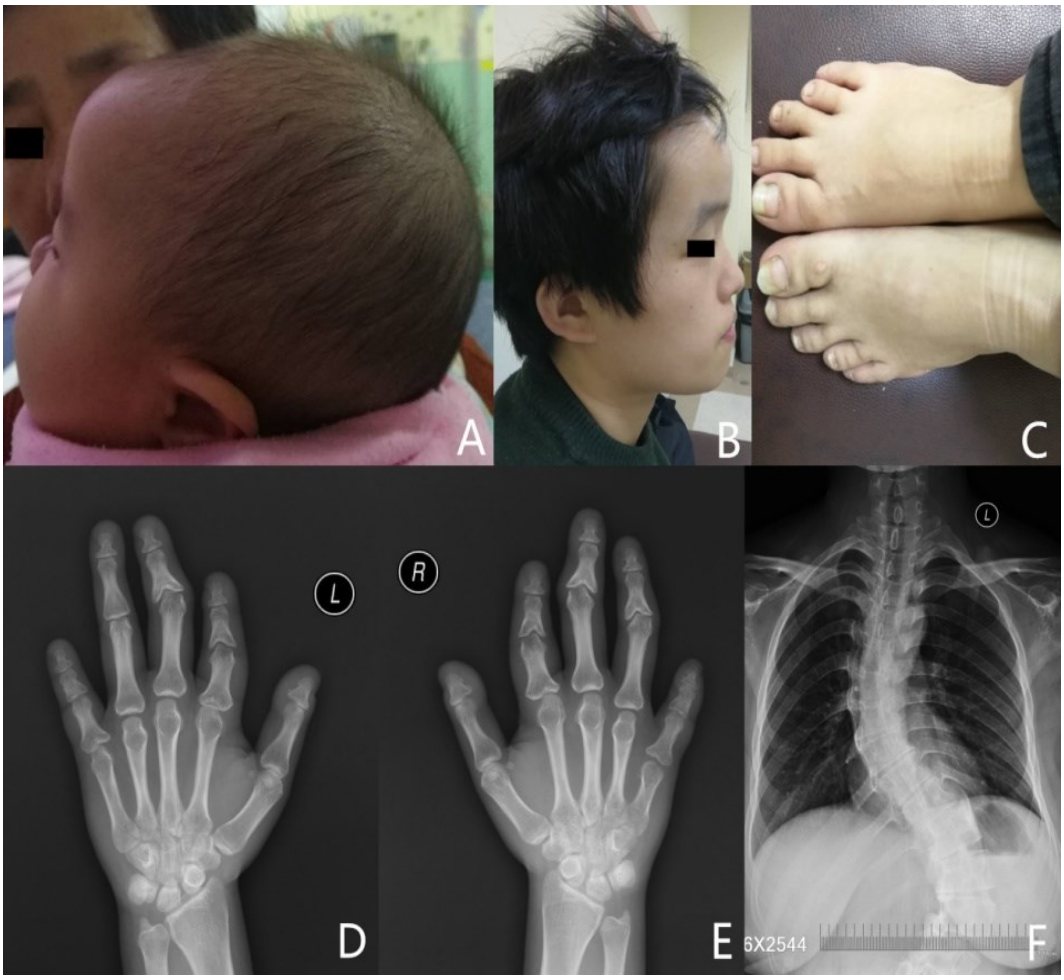

Figure 1. Clinical manifestations of proband and her mother A: Proband. B: Proband' mother. C: Feet of Proband' mother. D, E: x-ray plain films of the proband mother's hands. F: x-ray plain films of the proband mother's vertebral column.

3. Discussion

A total of 63 related articles were retrieved by searching the PubMed database with 'Greig cephalopolysyndactyly syndrome'and 'GLI3' as keywords from database beginning to February 28, 2019. According to clinical phenotypic study of GCPS, Raposo[2] found that the typical phenotypes include macrocephaly with frontal bossing, hypertelorism, and polysyndactyly. Although phenotypically diverse regarding the degree of polydactyly, more often than not polysyndactyly is postaxial in the hands and preaxial in the feet[4], accompanied by broad hallux or thumb and complex syndactyly in the limbs (Table 1). By analyzing GLI3 gene mutation data of GCPS in literatures[2,3,5-14]，common mutation types were summarized: frame shift mutations, nonsense mutations, missense mutations, splicing mutations, complete deletions and large deletions were 34%, 17%, 14%, 8%, 7%, 13% and 5%, respectively.

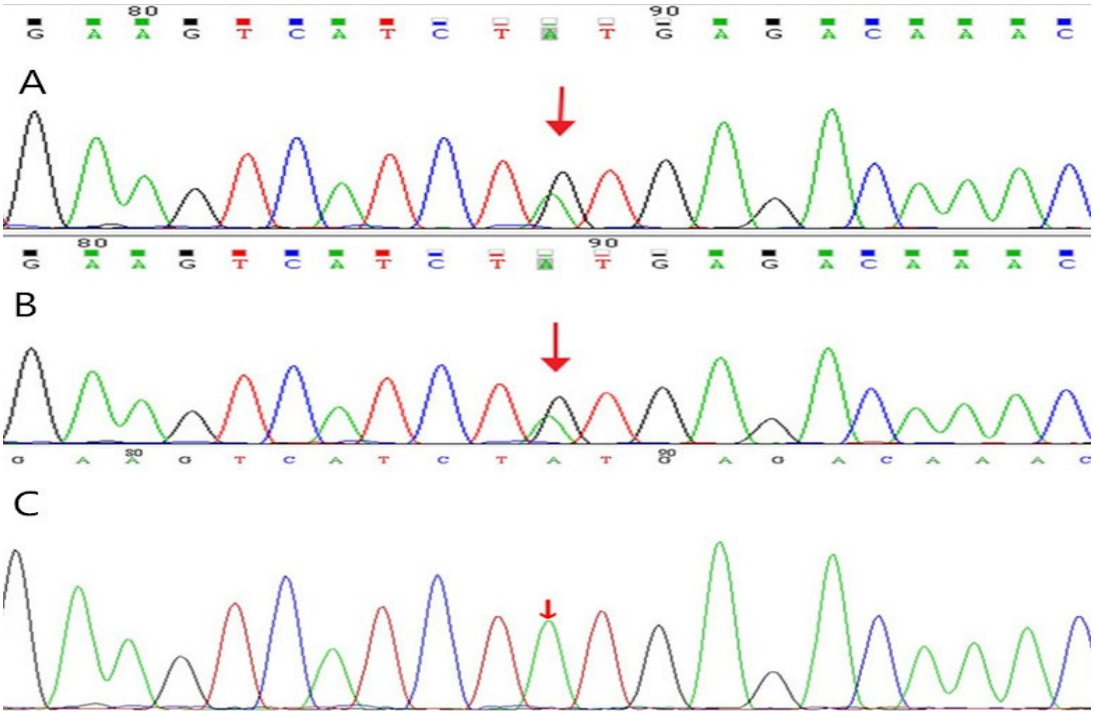

Figure 2. GLI3 gene sequencing map of proband and her parents. A: GLI3 gene of Proband has c.1433A>G (p.Y478C). B: GLI3 gene of Proband's mother has c.1433A>G (p.Y478C). C: GLI3 gene of Proband's father is normal.

Table 1. Incidence of typical abnormalities in GCPS

Abnormalities		Incidence
Craniofacies	Macrocephaly	52%
	High forehead	70%
	Frontal bossing	58%
	Broad nasal root	79%
Hands	Postaxial polydactyly	78%
	Broad tumbs	90%
	Syndactyly(primarily in finger 3 and 4)	82%
Feet	Preaxial polydactyly	81%
	Broad halluces	89%
	Syndactyly(primarily toes 1 to 3)	90%

The GLI family zinc finger protein are transcriptional effectors of the canonical hedgehog (HH) signaling pathway. GLI participates the regulation of gene expression and inhibition in various stages of embryonic morphology[15]. GLI3 is one of the three transcription factors in the GLI family and is an essential regulator of normal tissue development. GLI3 has several highly conserved functional domains, including an N-terminal transcriptional repressor and two C-terminal transcriptional activation domains, endowing GLI3 with dual and opposing functional capabilitie[15]. In the central region of GLI3, there are five C2H2-type zinc finger domains that mediate DNA binding, and a domain that mediates GLI3 proteolytic processing[9]. In the absence of HH signaling pathway, GLI3 is processed into N-terminal transcription inhibitor (GLI3R). When HH signaling pathway is activated, GLI3R formation is blocked and converted into transcriptional activator (GLI3A)[9]. GLI3A promotes HH target gene expression together with other GLI transcription factors (GLI1 and GLI2). Therefore, maintaining a proper balance between GLI3 activator and truncated GLI3 repress is essential for the normal development of tissues and organs[16]. Mutations in different domains of GLI3 gene can lead to different congenital diseases, including Greig cephalopolysyndactyly syndrome (GCPS), Pallister-Hall syndrome (PHS), non-syndromic Postaxial polydactyly typeA1 and B(PAP-A/B) and preaxial polydactyly type IV (PPD-IV)[17].

The specific clinical diagnostic criteria for GCPS are not yet clear. There are a large number of different GLI3 gene mutations in GCPS, so the clinical phenotypes vary and lack specificity. The typical clinical manifestations of GCPS are macrocephaly with frontal bossing, hypertelorism, and

polysyndactyly. Other uncommon abnormalities include corpus callosum dysplasia, mild hydrocephalus, heart defects, umbilical hernia, cryptorchidism, hypospadias, craniosynostosis, and cognitive impairment[2]. But not all patients with GCPS have craniofacial deformities or polysyndactyly. Sethi[12] reported two different families of GCPS, none of which showed characteristic craniofacial deformities, and only polydactyly in varying degrees. However, the two families were definitely diagnosed as GCPS by GLI3 gene analysis. The severity of the polydactyly varies among individuals and even among different limbs in the same individual. This can vary from an apparently normal extremity, through subtle broadening of the thumb or hallux, tiny postaxial nubbins, to partially bifid digits, hypoplastic supernumerary digits, fully formed supernumerary digits, and higher order polydactyly[12]. Therefore, even if the clinical phenotype of patients does not conform to the typical GCPS manifestations, we should screen individuals with familial polydactyly or similar craniofacial abnormalities for GLI3 gene detection. Phenotypic variations in some GCPS patients are severe, mostly due to a large number of deletions in GLI3 gene[18]. In cases of complete or massive deletions of GLI3, patients may have overlapping phenotypes with acrocallosal syndrome. In addition to craniofacial abnormalities and polydactyly, there are also callosal dysplasia, cognitive impairment and epileptic seizures in those GCPS patients. With the increasing application of gene detection in clinical diagnosis, new mutations are constantly detected, and new phenotypes may appear at the same time. A recently case[11] of GCPS was detected a novel mutation, c.2155 C> T (p.P719S) in GLI3 gene, which triggered the overlapping phenotype of GCPS and PHS, accompanied by gallbladder and pancreatic dysplasia. The rare phenotypes of GCPS, such as gallbladder and pancreatic dysplasia, can not be ruled out as being related to this new mutation.

4. Conclusion

The clinical diagnosis of GCPS is challenging. Johnston proposed a more relaxed presumptive diagnosis, including at least one limb of the polydactyly or abnormal wide thumb or hallux, varying degrees of syndactyly, macrocephaly and widely spaced eyes. In this GCPS case, the mother of the proband in the family had curvature of fingers and vertebral column. Whether the phenotype of skeletal dysplasia is related to the new mutation in GLI3 gene of GCPS remains to be further studied. It is suggested that the child with GCPS in this case should regularly monitor the growth rate of head circumference, pay close attention to the development of fingers and vertebral column, and also pay attention to the psychomotor development.

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