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Gene analysis: A rare gene disease of intellectual deficiency-Cohen syndrome

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ABSTRACT

Cohen syndrome is a rare, genetic, connective-tissue disorder, which is caused by mutations in the gene COH1 (VPS13B, Vacuolar Protein Sorting 13 Homolog B) at the chromosome 8q22. The disease is rare reported, which major clinical features include postnatal microcephaly, obesity, short stature, intellectual disability, progressive retinal dystrophy, intermittent neutropenia and many other unusual facial feature. We report four patients in China who were diagnosed with Cohen syndrome by genetic testing and clinical manifestations. At the same time, we review the related literature, and further expound the molecular mechanism of the disease, a variety of clinical manifestations, treatment and prognosis.

1. Introduction

Cohen syndrome, as one of the rare autosomal recessive disorders, was first reported in 1973 by (Cohen et al. (1973)). VPS13B (Vacuolar Protein Sorting 13 Homolog B) is a causative gene of Cohen syndrome. The typical phenotype of Cohen syndrome is variable and includes mild to severe psychomotor retardation, microcephaly, a cheerful disposition, characteristic facial features, childhood hypotonia and joint laxity, truncal obesity, intermittent neutropenia, along with a progressive retinal dystrophy and refractive myopia (Mehryar Taban and Dina et al., 2007).

Up to now, in addition to the above symptoms, other rare clinical manifestations were more and more reported, include pulmonary arterial hypertension, insulin resistance, thyroid and genital defects, diabetes, febrile seizures and epilepsy and skin problems (Cokkinos et al., 2013; Atabek and Keskin, 2004). Recently, Akihiro Abe et al. indicated that rearrangement of VPS13B could be a candidate for additional genetic events in variant t(8;21). Disruption of VPS13B may cause the unusual features of RUNX1–RUNX1T1 leukemia (Abe et al., 2017). With the development of genetic test technology, more and more novel VPS13B mutation were reported. Cohen syndrome has been assigned to mutations in the VPS13B gene. About 199 VPS13B mutations were reported up to now(Human Gene Mutation Database). Hennies et al. reported 17 novel VPS13B mutations among an ethnically diverse group of patients who have characteristic clinical manifestations (Hennies et al., 2004). And Rejeb et al. reported a Tunisian family including two siblings with developmental delay and intellectual disability harbouring a novel compound heterozygous mutation in the VPS13B (Rejeb et al., 2017).

We reported four cases who were diagnosed with Cohen syndrome by clinical manifestations and genetic technology. The purpose of this report is to analyse the clinical characteristics of Cohen syndrome and review related literature. This can make us better to diagnose this disease early. At the same time, this can improve the targeting of gene diagnosis.

2. Materials and methods

2.1. Subjects

The 4 Chinese children were gathered from the Affiliated Hospital of Qingdao University and the Qingdao Women & Children's Hospital.

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2.2. Sample collection

We collected 2 ml vein blood from each patient and from each inherited parent of the subject separately with the signed informed consent. Then the blood samples were sent to Beijing Kangso Medical Inspection for gene test of intelligence and motor developmental delay diseases, including VSP13B gene testing.

2.3. Genomic DNA extraction

The Qiagen FlexiGene DNA kit (Qiagen company, German) was used to extract genomic DNA from blood samples, following the guidance of manufacturer. The genomic DNA extracted from blood specimen was set for storage under −20 ℃. The PCR reaction procedure was as follows: 95℃ for 10 min, 35 cycles (95℃ for 30 s, 60℃ for 30 s, 72℃ for 45 s) followed by a final extension step at 72℃ for 5 min.

2.4. DNA library construction

To construct the DNA library, genomic DNA sample was fragmented into 150 ~ 300bp DNA fragments by ultrasonic processor. Adaptors to both ends of these DNA fragments were ligated and cohesive ends of the DNA fragments were trimmed. The DNA library was amplified and purified by PCR.

2.5. Hybrid capture

The target DNA fragments from amplified DNA library were hybridized and captured by probes and then amplified through SureSelect target enrichment system (Agilgent). Then the products were purified and quantified.

2.6. Sequencing

Single-read sequencing was performed by NextSeq500 (illumina). Raw data were acquired in the format of Fastaq.

2.7. Data analysis

Raw data can be transformed into identifiable base sequence with software CASAVA (1.8.2). Then Align analysis, SNP analysis and DIP analysis were conducted to obtain information of mutation sites from targeted region. At last, protein damage analysis was conducted to qualitatively predict the probability of the results by PolyPhen-2.2.2, and thus obtain ingmutation sites which need further validation.

2.8. First-generation sequencing verification

The gene sequences of above variation sites were acquired from GenBank. The primers were designed by the website Primer Z(http://genepipe.ncgm.sinica.edu.tw/primerz/primerz4.do)and then synthesized. The variation sites were amplified using PCR and sequenced with the first-generation sequencing. The obtained sequences were aligned with the previous results, and false positive sites obtained by NGS (the next generation sequencing) were ruled out.

3. Case report of four patients

3.1. Patient 1

A 2-years-old male saw a doctor because movement function development fell behind for 2 years. He had special features, including small head circumference(occipitofrontal circumference was 43 cm), sparse eyebrows, mild oblique palpebral fissure. His language development fell behind seriously, only could speak single-tone like “ba, ma”. He could not sit alone when 9 months of old, could not walk alone until now. The limbs had muscle weakness and hypotonia. The previous constitution was poor, easy to catch a cold. He suffered from intermittent neutropenia (neutrophils count 1.03×10^9/L). Maybe that's why he is easy to get infected. Brain MRI showed brain parenchyma owed to the full, subarachnoid space of basilar cistern, anterior longitudinal fissure and bilateral frontotemporal widened. Because the boy had special facial features, both movement and intelligence development fell behind seriously, we completed gene test of intelligence and motor developmental delay diseases to explicate whether he had genetic mutations. The results showed VPSl3B gene mutation: c.5086 C > T, inherited from his father; c.6940 + 1 G > T, inherited from his mother, both were heterozygous mutations, so the boy was diagnosed with Cohen syndrome by genetic testing and clinical manifestations. After given family rehabilitation therapy, the boy can walk alone now.

3.2. Patient 2

Another 5-years-old male was admitted to our clinic because of repeated convulsion for 5 days. The pattern of convulsion showed two eyes staring, mouth around cyanosis, limbs rigidity jittering, which occurred spontaneously and lasted for 3–5 minutes, which was a generalized seizure. In the most serious period, tic once every 10 min. Given valproate to control convulsions, poor effect, combined with clonazepam, control was still poor. He was the second child of nonconsanguineous parents, whose mother had aborted twice for unknown reasons. He was born after 38 weeks gestation with a birth weight of 2700 g. No problems were observed during the neonatal period. He achieved head control at 4 months of age, sat at 6 months of age, and walked independently at 15 months of age. His occipitofrontal circumference was 50 cm, which is normal according to his age. No difference was found about these aspects between this boy and normal children, but this boy was easily agitated. The language development and the intelligence was not very normal, but has no serious effect on the daily communication. About facial condition, he was normal in general. His brother was a very health boy up to now. No abnormalities were found during the routine laboratory investigations. Metabolic screening, electroencephalogram and cranial magnetic resonance imaging were normal. Because the epilepsy was not easily controlled and his intelligence was lower than normal children, we completed the relevant genetic check. Genetic analysis results showed that two heterozygous mutation were found in the exon region of VPS13B:c.3203C > T and c.8016 + 7G > C. The c.3203C > T was heterozygous mutation inherited from the father. The c.8016 + 7G > C was heterozygous mutation inherited from the mother. The mutation site of c.3203C > T missense mutation caused change in amino acid, p.Thr1068Ile.

3.3. Patient 3

Another 3-years-old boy came to our hospital because of developmental and mental retardation. Through physical examination, we found that the boy had small head circumference(occipitofrontal circumference was 47 cm), low muscle tone and low tendon hyperreflexia. We completed the intelligence relevant genetic check. Compound heterozygous nucleotide variants were found in VPS13B gene: c.2199C > A and c.553 T > C. The c.2199C > A leads to compile the 733th amino acid Cys codon to termination codon (p.Cys733Ter), so that the peptides synthesis was early terminated, as a nonsense mutation. The c.553 T > C change the 185th amino acid from Trp to Arg (p.Trp185Arg), as a missense mutation.

3.4. Patient 4

The forth boy who also had small head circumference, low muscle tone and low tendon hyperreflexia came to our hospital because of hypophrenia and developmental retardation. He was 3-years-old and

Table 1
The detailed clinical manifestations, relevant check results including genetic detection of the four patients.

Patient number	1	2	3	4
Chief complaint	The movement function development fall behind for 2 years	Repeated convulsion for 5 days	Developmental and mental retardation	Hypophrenia and developmental retardation
Gender	boy	boy	boy	boy
Age	2 years old	5 years old	3 years old	3 years old
Height	76cm	108cm	100cm	86cm
Weight	11kg	22kg	16kg	11kg
Head Size	43 cm	50 cm	47 cm	43.5 cm
Facial Features	Sparse eyebrows, mild oblique palpebral fissure	No special	Narrow forehead, micrognathia	Narrow forehead, oblique palpebral fissure
Language Development	Language development fall behind, only can speak single-tone	Language development was not very normal, but has no serious effect on daily communication	Only can call mother, understand simple instruction	Language development fall behind
Gross Motor Development	Cannot sit alone when 9 months, cannot walk alone until now	Walk independently at 15-month-old	Sat independently when 6-month-old,walk independently nearly 2-year-old	Walk independently 2-year-old
Motor function	Low muscle tone and low tendon hyperreflexia	Normal	Normal muscle tone and low tendon hyperreflexia	Low muscle tone and low tendon hyperreflexia
Birth History	No special	Mother have aborted twice for unknown reasons	No special	No special
Past medical history	Easy to catch a cold	Easy to catch a cold	No special	No special
Family History	No special	No special	No special	No special
Blood Routine Examination	Neutrophils count 1.03×10^9 / L	Normal	Normal	Normal
Blood Glucose	Normal	Normal	Normal	Normal
Thyroid Function	Normal	Normal	Normal	FT3 7.05 nmol/L (reference range 3.1-6.8 nmol/L), FT4 and TSH are normal
Brain MRI	Brain parenchyma owed to the full, subarachnoid space of basilar cistern, anterior longitudinal fissure and bilateral frontotemporal widened	Normal	Normal	Cranial capacity were small
Echocardiogram	Normal	Normal	Normal	Normal
Ophthalmic test	Normal	Normal	Normal	Normal
Gene Detection	c.5086C > T,c.6940 + 1G > T	c.3203C > T,c.8016 + 7G > C	c.2199C > A; c. 553 T > C	c. 6940 + 1 G > T; c.9852-9855del

the occipitofrontal circumference was 43.5 cm. The brain MRI showed that his cranial capacity were small. We also completed the intelligence relevant genetic check. Compound heterozygous nucleotide variants was found in VPS13B gene: c.6940 + 1 G > T and c.9852 _9855del. The c.6940 + 1G > T leads to the first bit of nucleotides in coding area after 6940 nucleotides introns mutate from G to T, as a shear mutation. The c.9852 _9855del refer to encoding lack of district no. 9852 _9855 nucleotide. This mutation causes the change of amino acid synthesis which start from the 3287th amino acid(Glu amino acid), and terminate after the change of 28 amino acid (p.G lu3287AlafsTer28), as a frameshift mutation.

The mutation locations in VPS13B gene of the four cases are different from each other. The four patients are all boys and everyone has different intelligence retardation. Three patients has special facial characters like narrow forehead. One patient suffer from repeating convulsion and was diagnosed clinically as epilepsy. Other three patients were perplexed by intelligence and motor developmental delay. Two patients had brain structural abnormity. One patient suffered from intermittent neutropenia. The number of neutrophils in the other three patients is normal (Table 1).

4. Discussion

4.1. Molecular studies

Cohen syndrome, as a rare autosomal recessive disorders, is caused by mutations in the gene

COH1 (VPS13B). VPS13B is described as the single Cohen Syndrome linked gene up to now. It is localized on q22.2 locus of chromosome 8. Its length is about 864 kb and comprises 62 exons. The longest transcript [NM_017890.4] is 14,100 bp long encoding for a 4022 amino acid. Study has clearly defined COH1 as a Golgi-enriched scaffold protein that contributes to the structural maintenance and function of the Golgi complex (Seifert et al., 2011). The main cellular functions of the Golgi apparatus include protein sorting and packaging and the post translation modification, such as glycosylation of newly synthesized proteins and lipids. COH1 is critical for the orientation of the Golgi toward the longest neurite. Positioning of the centrosome and the Golgi complex in postmitotic neurons determines the accelerated outgrowth of one neurite by which the prospective axon is specified (Zmuda and Rivas, 2010). Wenke Seifert et al. have reported COH1 regulates the differentiation and integration of neurons into a functional network. Reduced COH1 attachment to the Golgi membrane will inhibits efficient polarization and targeted membrane transport toward the

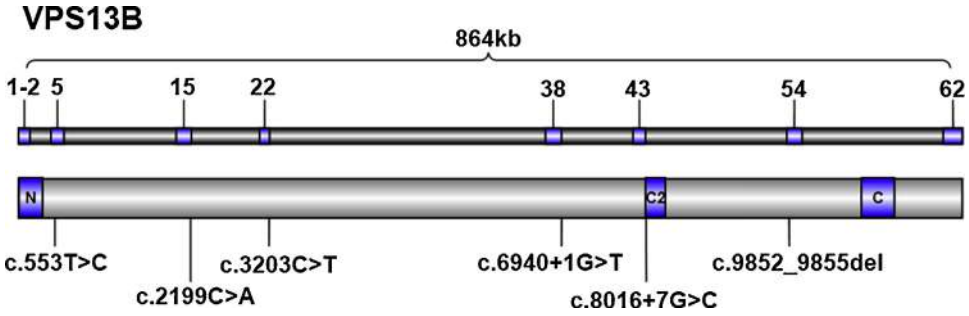

Fig. 1. VPS13B gene structure and locus position.

developing axon (Seifert et al., 2015). Laurence Duplomb et al. have verified that VPS13B plays a major role in the function of the Golgi apparatus associated with major alterations in protein glycosylation in CS patients with VPS13B mutations (Duplomb et al., 2014). Abnormal protein glycosylation or disturbance of the Golgi function are responsible for neutropenia, truncal obesity, retinopathy, intellectual deficiency, etc.

According to the Human Gene Mutation Database, about 199 VPS13B mutations were reported to date. Whether homozygous or compound heterozygous mutations, they resulted in shorter or abnormal proteins. We report here four VPS13B mutations including missense mutation, nonsense mutation and move code mutation, thus enlarge the VPS13B mutational spectrum. We hypothesized that amino acid change lead to abnormal protein structure, which can influence the function of the neuron, then lead to disease (Figs. 1 and 2).

4.2. Clinical manifestations and diagnosis

The clinical manifestations included microcephaly, primary cutis verticis gyrata of the scalp, prominent supraorbital ridges, coarse facial features (large nose, hypertelorism), retinitis pigmentosa, cataracts, sensorineural hearing loss and kyphoscoliosis in addition to mental retardation (Megarbane et al., 2001). Once a study has analysed the evolution of CS facial features in the early period of life to find clues for an earlier diagnosis, and then select accurate genetic counselling (El Chehadeh-Djebbar et al., 2013). Horn et al. have ever proposed a criteria in 2000 to diagnose the Cohen Syndrome, which requiring patients to have at least three major signs and one minor sign of the disease, major signs including mental retardation, short stature, hypotonia, microcephaly, chorioretinal dystrophy, narrow hands and feet, minor signs including truncal obesity, neutropenia, myopia, minor facial anomalies of short philtrum, prominent upper incisors, thick eyebrows, micrognathia, high arched palate, and maxillary (Horn et al., 2000). One newly diagnostic guideline was given in which a child with significant learning difficulties must show at least two of the three features: typical facial gestalt, pigmentary retinopathy or neutropenia (Chandler et al., 2003; Douzgou and Peterson, 2011). But not criteria recognized by internation was accepted. Cohen syndrome, due to the location of the gene mutation and the amount of mutations, lead to illness from mild to serious thus early diagnosis become difficult. Dastan J et al. detected that some CS features like Truncal obesity and spine abnormality are age-dependent and evolve later in childhood. This character also increases the difficulty of diagnosis (Dastan et al., 2016). The incidence of epilepsy in Cohen syndrome is 6%. Gueneau L et al. reported a case of congenital neutropenia associated with retinopathy, even in the absence of intellectual deficiency (Gueneau et al., 2014). In our reseach, one patient suffered from intermittent neutropenia. The number of neutrophils in the other three patients is normal. Neutropenia is a clinical sign important in Cohen syndrome. But in our research, this clinical charactor is not universal in all patients diagnosed as Cohen syndrome. These unusual features extend the VPS13B phenotype spectrum. With the development of gene diagnosis technology and precision medicine, when clinical reception of psychomotor retardation cases, we should consider the Cohen syndrome, and perfect genetic testing as early as possible. The perfect accompaniment of the clinical diagnosis and genetic diagnosis can promote early diagnosis.

4.3. Treat and prognosis

Cohen syndrome refers to an autosomal recessive syndrome of multiple congenital anomalies with mental retardation (Balestrazzi et al., 1980). The clinical features of Cohen syndrome vary as the disease progresses over time. A multidisciplinary approach to treatment that includes a pediatrician, medical geneticist, ophthalmologist, psychologist, physical and speech therapist, dentist, and endocrinologist ensures the best possible lifestyle and health (Mehryar Taban and Dina et al., 2007). No effective treatment has been developed to halt the progression of the retinal disease to date. The main treatment is symptomatic and supportive treatment, especially the rehabilitation. If convulsion occur in Cohen syndrome patient, children should be actively given effective anti-epileptic drug therapy. At the same time, nutritional education should also be brought to the forefront because diabetes are common in Cohen Syndrome which is an important risk factor of cardiovascular disease. Family rehabilitation therapy is also necessary for these patients and more love should be given to them. As an autosomal recessive syndrome and the poor prognosis, genetic counseling is essential.

5. Conclusion

We report four cases of Cohen Syndrome in China. When reception of patients intellectual deficiency with epilepsy, special face with hypophrenia and delayed motor patients, we need to consider the possibility of the disease and complete genetic tests as soon as possible, because early diagnosis, early symptomatic support treatment, may improve the prognosis. Prevalence of Cohen syndrome remains unknown because only about 250 cases have been reported worldwide (Atabek and Keskin, 2004). More and more special manifestations also have been reported with the development of gene testing. Maybe this gene has connection with facial features, material metabolism and hormone secretion. Because of the different mutation locations, the clinical manifestations are different from each other, but most have intelligence and movement retardation. This may be related to the primary function of the VPS13B gene. The mutation of this gene change the function of nerve conduction by altering the protein construction and function. The molecular mechanism how mutations in VPS13B affect the various clinical manifestations need to be investigated further.

Conflicts of interest

None.

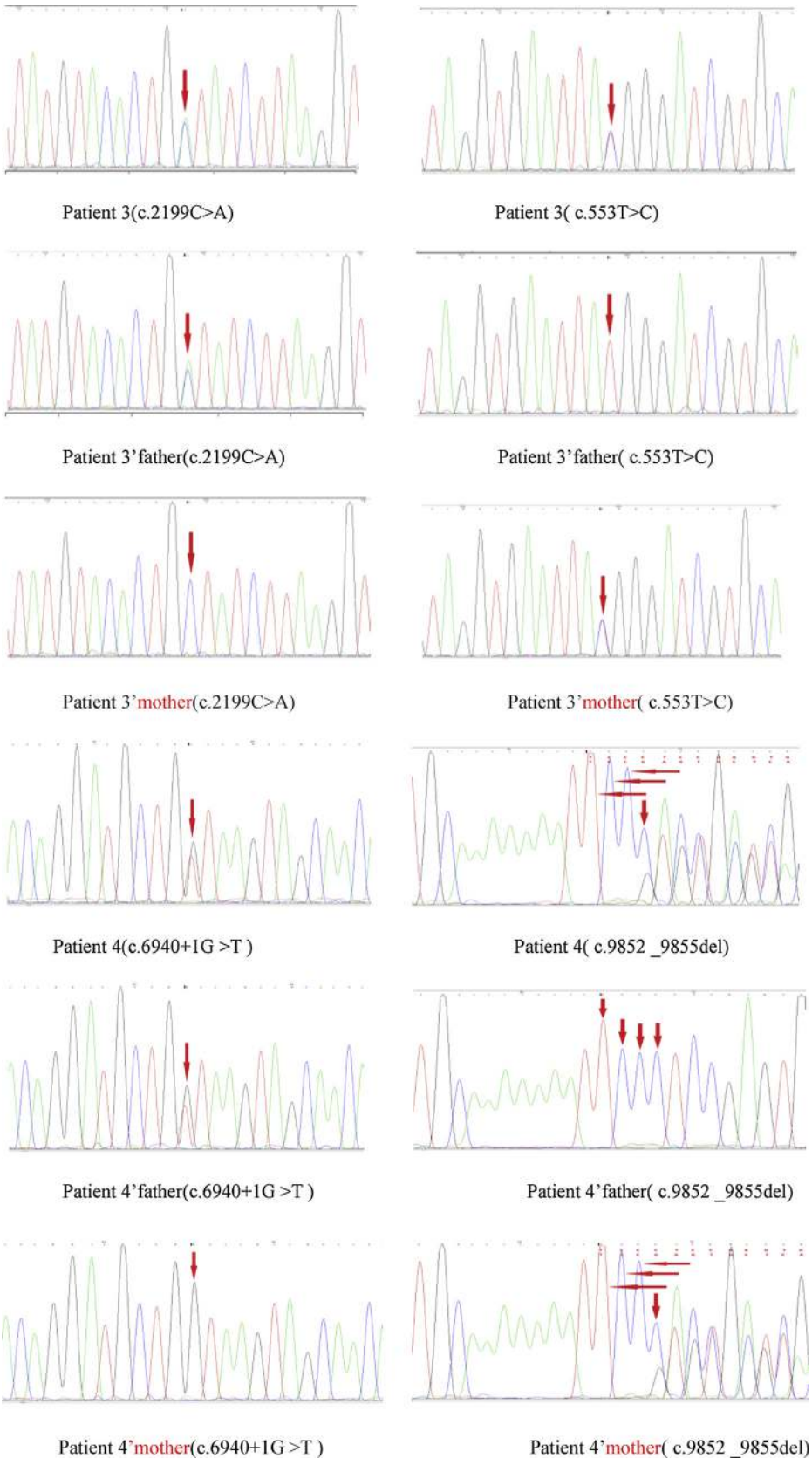

Fig. 2. The sequence chromatograms of heterozygous mutations in VPS13B gene.

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