Neonatal Oculocutaneous Albinism Type 2 With Prader-Willi Syndrome

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Case Report

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Abstract

Background: To investigate the pathogenesis and clinical characteristics of Oculocutaneous albinism type 2 (OCA2), a genetic condition in the etiology of Prader-Willi syndrome (PWS).

Case presentation: A retrospective study of one case presented with poor response to stimuli, difficult

feeding, poor crying, with yellow hair and white skin. We performed genetic testing and investigated disease pathogenesis, clinical manifestations, and diagnosis, and discussed the characteristics of the disease through a literature review. HiSeq high-throughput sequencing result suggested a deletion with 105 genes, including UBE3A, SNRPN, OCA2, and other genes up to 5.18 Mb on the long arm of chromosome (15q11-13 region), a critical region, susceptible to the PWS. A paternally derived deletion Del (15q11. 2q13. 1) [GRCh37 / hg19] (23, 378, 392-28, 563, 050) × 1, and a maternal missense mutation were identified in the *OCA2* gene (chr15: 28171296 c .2056G> A (p.A686T). During the period of hospitalization, the child still suffered from poor milk intake, and she was discharged from the hospital at the request of her parents. After discharge, the patient was followed up for two months by telephone. However, the patient died of feeding difficulties and pulmonary infection.

Conclusions: OCA2 combined with PWS due to OCA2 gene missense mutation combined with large fragment deletion of 15q11-13 region was first reported in this study, of which the clinical signs can be subtle and symptoms can be more severe, therefore, early genetic testing is crucial for those patients to yield an accurate diagnosis and initiate aggressive interventions to optimize the outcomes.

Background

Albinism refers to a group of autosomal recessive genetic diseases. Oculocutaneous albinism type 2 (OCA2) is the most common form of albinism and prevails worldwide [1]. OCA2 is characterized by reduced pigmentation of a few of the major organs of our body including, the skin, hair, iris, and retina. Individuals diagnosed with OCA2 are born with minimal to near-normal cutaneous pigmentation [2]. Genetically, OCA2 is a heterogeneous autosomal recessive disorder and caused by point mutations or deletions of the oculocutaneous albinism (*OCA)* gene, located within the Prader-Willi syndrome (PWS) critical region of 15q11.2-q13.1, which is subject to genomic imprinting, the process by which certain genes are activated or inactivated depending on their parent of origin [3]. PWS, also known as hypotonia-mental retardation-gonad development lag-obesity syndrome, is characterized by severe speech impairment, developmental delays, hypotonia, feeding difficulties, and behavioral features, including frequent laughing [4].

OCA2 is particularly common in North America and Europe, with an overall incidence rate of 1/17000 [5], and it is most frequently observed in Africa. More common in clinical practices, OCA can be categorized into different sub-types based on the color of the eyes, skin, hair, and pathogenic genes. Currently, there are seven different types of OCA reported.

With the development of genetic diagnostic technology, accurate diagnosis is possible for rare diseases like OCA and PWS. Multiplex Ligation Dependent Amplification (MLPA) is an advanced, and new gene quantification technology with an advantage of repeating, deleting and quantifying multiple genes, and exons at the same time [6]. Besides, Next Generation Sequencing (NGS) technology is highly sensitive with high throughput capabilities and offers a short experimental cycle. With the help of these advanced technologies, locating new genes and analyzing gene mutation types and mutation size can now be accomplished efficiently, quickly, and precisely [7]. MLPA, combined with NGS, can efficiently detect gene deletions, duplications, and minor mutations and makes the clinical diagnoses more efficient and accurate. These technologies are now widely used in the diagnoses of rare clinical diseases.

This study reports a case of OCA2 with PWS, where the gene mutation was diagnosed by using MLPA and NGS technology, and its family. Results from this study provide clinical insights of OCA2 and PWS, and offers new ideas for clinical diagnosis.

Case Presentation

Materials

The proband, a 6-hour-old girl, G_2P_2, was born at 40+1 weeks gestation in a natural delivery. The Apgar score was 10'-10', with a birth weight of 2700g, body length of 47 cm, head circumference of 34 cm, and chest circumference of 31cm, and the amniotic fluid pollution II°. Maternal pregnancy had "gestational diabetes and hyperthyroidism." The first child was a 5-year-old boy with yellow hair, without any other family history of hereditary diseases. The proband had feeding difficulties, hypotonia, poor crying after birth, crooked mouth, and born with yellow hair and white skin. Physical examination: yellow hair and white skin, plain and soft anterior fontanel with about 2.0×2.0 cm size, a narrow bifrontal diameter, small fissure, strabismus, a small upturned nose with a thin upper lip and down-turned corners of the mouth, high palatal arch, sticky salvia and a dry mouth with enamel hypoplasia, soft neck, no abnormality in heart, lung and abdomen examination, normal limb movement, the left testicle was not touched, hypotonia, negative pathological signs. The ophthalmic fundus examination showed changes in albinism (Figure 1). After obtaining the informed consent from the child's parents, peripheral venous blood was collected, and DNA was extracted for genetic testing. During the period of hospitalization, the child still suffered from poor milk intake, and she was discharged from the hospital at the request of her parents. After discharge, the patient was followed up for two months by telephone. However, the patient died of feeding difficulties and pulmonary infection.

Method

Genomic DNA uptake: Anticoagulant blood collection tube containing EDTA was used to collect 5 ml of peripheral venous blood from proband and related family members, and stored at 4°C. DNA was extracted using a 96-channel automatic nucleic acid extractor (Cat. No: CWE9600. China's high-end biological reagent manufacturer, Beijing, China) and associated Blood DNA Kit CWE2100 V2 (Cat.No.CW2553, China's high-end biological reagent manufacturer, Beijing, China).

High-throughput sequencing: After the DNA was extracted by using the ultrasound technique, the DNA sequencing library was constructed by using an exon chip (Agilent. The easy life, Shanghai, China) and HiSeq high-throughput sequencing (Illumina, Back far gene, Nanjing, China). The sequencing results were compared to the GRCh37 / hg19 standard sequence, and the sequence was analyzed for possible mutations. For pathogenicity prediction and point mutation analysis, HGMD Professional Edition database, Thousands of Human Genomes, gnomAD, ExAC database, dbSNP database, SIFT, PROVEAN, PolyPhen-2, and Mutation Taster (MyGenostics, Beijing, China) database was used. The predicted data were combined with the probang's phenotype and pedigree analysis by following the ACMG Classification Criteria for pathogenicity.

Multiplexed Probe Amplification: Based on the results of high-throughput sequencing, the SALSA MLPA Kit (Mlpa mrc, Beijing, China) was used to amplify and detect intervals suspected for CNV mutations.

Sanger sequencing verification: Based on the high-throughput and multiplexed ligation probe amplification sequencing results, Sanger sequencing was used to verify the mutation results of the proband and their relatives. For mutations in the OCA2 gene and OMIM gene, Primer3 Input (http: //Primer3.ut.ee/) was used to design primers with the following sequences: Forward primer 5'-GCTGTCAGAAATCTCTCAGTGG-3', Reverse primer 5'-AAAGAAAAAGGCAACCAGAGG-3'. AB3730XL (Shanghai zhiyan scientific instrument, Shanghai, China) was used to amplify the mutation site region with a specific primer. The DNA analyzer was used for DNA sequencing, and the sequencing results were compared to the standard sequence (5'-AAGAGCGCTGCAAAAAACAFA-3').

Statement

Informed consent was obtained to publish the information/image(s) in an online open access publication.

Results

Gene sequencing

Gene sequencing results revealed a maternal heterozygous missense mutation at the OCA2 gene chr15: 28171296 c.2056G> A (p.A686T; NM_000275; Figure 2). This mutation was located at the transmembrane helix region of the OCA2 gene. Consistent with our finding, multiple pathogenic mutations have been reported near this site in previous studies [8], and are likely to affect its function (PM1: Located in hot spot mutation regions and/or in key functional domains (such as active sites of enzymes) without known benign mutations). It is noteworthy that the mutation is neither detected in a healthy population nor available in the public

database (PM2: Extremely low-frequency loci in recessive diseases). The mutation site was predicted to be harmful (PP3: Various statistical methods predicted that the mutation would have harmful effects on genes or gene products) as detected and replicated by using different analysis platforms (SIFT, PROVEAN, PolyPhen-2, and Mutation Taster). The fundus examination of the participant revealed OCA. During analysis, this was the only mutation detected in the participant (PP4: The phenotype or family history of the carrier of the mutation is highly consistent with a single genetic disease). Besides, the mutation site is highly conserved among different species (Figure 3). According to the ACMG code, the mutation position was found to be pathogenic (Likely Pathogenic = PM1 + PM2 + PP3 + PP4). Mutation analysis revealed a chromosomal heterozygous deletion (15q11. 2q13. 1) [GRCh37 / hg19] (23, 378, 392-28, 563, 050) × 1, fragment size: 5.18 Mb, in the participant and was found to be of paternal origin. The deletion interval included at least 105 essential genes, including UBE3A, SNRPN, and OCA2, which are reported to be the critical markers of OCA (Figure 4) [9]. Moreover, the deletion of 15q11.2 locus was reported to be overlapped with PWS. This observation is supported by the fact that multiple patients with PWS had inherited deletion mutations spanning the NDN or SNRPN region, which originated from the paternal side. Our observation is consistent with these reports, where the deletion mutations in the probang were found to be covered by the SNRPN gene. It is interesting to notes that this deletion also included the OCA2 region. We noticed a higher degree of variation among the two alleles of the probang's OCA2 gene (Figure 4). We speculate this mutation as the cause of OCA. Besides, the patient carried a chromosomal repeat mutation Dup (12q22) [GRCh37 / hg19] (94, 022, 544- 94, 624, 229) × 3, but the clinical significance of this variation is unknown, and no distinct characterization was found.

Family Survey and Family Map

According to the family survey, the father of the probang had growth retardation during childhood. However, no consequences of the growth retardation were observed in adulthood. Moreover, the father of the probang was never diagnosed with PWS. The probang's brother, mother, and grandmother had yellow hairs; Other family members were recorded to be healthy without any genetic defects. Parents were married to distant relatives. With this information, we combined the sequencing results to determine the genotypes of each family members and extrapolated a genetic map, as shown in Figure 5, below.

Discussion And Conclusions

OCA is an autosomal recessive rare genetic disease [1]. Patients with OCA have reduced or even lack melanin biosynthesis due to genetic defects [2]. Clinical reports suggest eye color of the OCA patients as gray, light brown, or reddish-brown, with a defect in visual acuity, and often accompanied by vision loss [10]. According to the clinical phenotype of the patient gene profile studies that cause disease etiology, OCA is categorized into seven different types; OCA1 through OCA7. Among them, OCA1 is further sub-categorized into OCA2, caused by a mutation in the OCA2 gene [11]. Patients carrying OCA2 mutations are reported to have a subtle change in the pigmentation, which progresses with age, and hence it is also called incomplete albinism or the classical tyrosinase-positive albinism. OCA2 mutation is most prevalent in African populations [12]. It is clinically characterized by hypopigmentation of the skin, hair and involves unique eye changes of all types of albinism: including nystagmus, the translucency of iris, and reduction of iris pigmentation, reduction of retinal pigmentation and clearness of choroidal vessels in fundus examination. It has been reported that the foveal dysplasia and misrepresentation of optic fiber radiation in the optic chiasma lead to strabismus, reduced stereo vision, and changes in the power generation position [13].

The OCA2 gene is located on chromosome 15 and encodes for transporter protein P on the melanosome membrane. The P protein is an 838-aminoacid long polypeptide chain foldeand modified, and its three-dimensional structure contains 12 transmembrane helixes [14]. These transmembrane helices together form transport support, which transports tyrosine across the membrane to melanocytes and provides a substrate for melanin catalysis by tyrosinase in the melanin body [15]. Currently, more than 300 cases have been recorded in the HGMD database depicting the pathogenic mutation of the OCA2 gene. Besides, patients with a heterozygous deletion mutation of *OCA2* experience a minimal or reduced synthesis of the transporter. OCA2 carries a semi-synthetic missense mutation c.2056G>A (guanine>adenine) on the remaining allele [16].This mutation substitutes alanine with threonine at 686th position of the 9th transmembrane helix, which results in the extension of the amino acid R group carbon chain [14]. This change significantly affects the spatial structure of the transporters, reduce or even prevent the transmembrane transport of tyrosine, and influence the synthesis of melanin. All these changes eventually lead to OCA phenotype [17]. Previous reports suggest that a small number of OCA2 gene mutations lead to brown hair or blue eyes [18]. However, this observation is

limited to missense mutations in non-coding regions or non-transmembrane helix regions of the coding regions. The mutations carried by the children, in this case, are located in the transmembrane helix regions, which may lead to OCA, and is consistent with our observation [19]. In this study, the mother, grandmother, and brother of the proband were heterozygous, and all three had yellow hair. We speculate that although the melanin was expressed in all three immediate relatives, the content was significantly less. This observation may explain the pathogenicity of the missense mutation.

PWS and Angelman syndrome (AS) are examples of imprinting defects which may be caused by a deletion, UPD, or imprinting failures. Clinical manifestations of AS include severe growth retardation, mental retardation, typical craniofacial appearance (round face and flat nasal bridge, up-slanted palpebral, epicanthic folds, etc), seizures, ataxia, language disorder, and mental retardation. If the 15q11-q13 deletion mutation affects the expression of paternally imprinted genes, it leads to PWS. The world's first PWS case was discovered in 1887, and was subsequently defined and named by Prader *et* al. in 1956 [20, 21]. Hundreds of PWS patients have been reported worldwide, and clinical features include: fetal movement decreases before birth; neonatal hypotonia, weakened reflect, difficulty feeding, and genital hypoplasia. Limb development occurs within 1 year of age, but eventually leads to uncontrolled food intake and centripetal obesity. PWS, also accompanied by growth retardation, and mental retardation, characteristic facial features (narrow long face, almond eyes, squint eyes, large chin, etc.), and reduced motion activities due to weak musculature. By the 6 years of age, patients develop itching and scratching, leave marks, abdominal olfactory lines, thick saliva in the corners of the mouth, insensitive to pain, diabetes, poor puberty development, and poor prognosis [4].

The PWS pathogenesis is categorized into five sub-types, 5-6Mb deletion of paternal alleles, which accounts for about 65% to 75% of all patients being the most common. PWS, then further sub categorized into type I and type II according to the location of the deletion. However, is no significant difference in the clinical outcome. PWS maybe the result of maternal uniparental disomy (UPD), with a relatively mild symptom, and accounting for 20% to 30% of the imprinted deletion, accounting for 2% to 5%; very few (<1%) are chromosomal imbalance shifts and single-gene mutations [22]. Studies have found that the loss of the imprinted gene NDN or SNRPN gene expression on chromosome 15 causes PWS the main reason [10, 11], but still does not rule out the possibility of other pathogenic genes in the 15q11-q13 interval. The NDN gene expresses the growth inhibitory protein Necdin, which is used in the nucleus to regulate the cell cycle, cell differentiation, and apoptosis. The expression of this protein is reduced or deletion can lead to unregulated cell growth regulation and cause disease [23]. The SNRPN gene can express two RNA-related proteins: the gene can express small nuclear ribonucleoprotein associated protein N (SNRPN protein) alone. Also known as SmN splicing factor), a type of Sm protein, is involved in the process of small ribonucleoproteins (snRNPs) shear modification of mRNA; at the same time, it can also be linked to a specific upstream section to express SNRPN protein and SNRPN protein upstream reading frame protein through a bicistronic transcript. (SNRPN upstream reading frame protein) [24]. Decreased or deleted expression of this gene is likely to affect the splicing and modification of mRNA, and then cause a series of neuroendocrine diseases. The chromosomal mutations carried by the children in this case also include the parent source of SNRPN Allele deletions have a high probability of causing PWS disease. Although the father of the child carries a mutation, the clinical phenotype is normal, it can be speculated that the chromosome deletion is a new mutation or inherited from the grandmother of the child, but the specific source of this mutation still needs to be further investigation and research.

There is a paternal deletion of the SNRPN gene in children with this study, which is in line with the genetic pattern of human genome imprinting disease PWS; the OCA2 gene. The gene deletion represents a compound heterozygous mutation of missense mutation and gene deletion, which is inconsistent with the autosomal recessive type 2 autosomal genetic method. Combined with phenotypes such as OCA and feeding difficulties, the diagnosis is OCA2 with PWS. Previously, 5 cases of children with PWS and other diseases have been reported in China. By analyzing the clinical and genotype statistics of these children, combined with the analysis of the children, in this case, we found that the compound diseases reported in children in China were all PWS-related complications. For example, diabetes, fatty liver, thyroid dysfunction, diabetic ketoacidosis, acute severe pancreatitis and other metabolic syndromes due to endocrine disorders in children, and acquired diseases such as scoliosis due to the obesity of children (Table 1), The child, in this case is the only child in China that has PWS and other diseases caused by congenital pathogenic genotypes, and the symptoms are more serious, and the child died during infancy.

The current work provides clinical insights into OCA2 and PWS and guidance for subsequent genetic counseling. Because albinism refers to a group of autosomal recessive genetic diseases, PWS is caused by the deletion of candidate gene expression

on the parent chromosome at chromosome 15 (15q11.2-q13.1), the father is carriers of multiple genes deletions and the mother is a carrier of the OCA2 mutation, so the patient is a 2nd child of his/her parents, it is most likely that the 3rd child will significantly be impacted. The probability occurrence of OCA2 and PWS is 1/8th, the probability occurrence of OCA2 alone is 1/8th, the probability occurrence of PWS alone is 3/8th. Genomic tests using either NGS or MLPA technology is highly recommended for any upcoming child if the parents are planning for a second child to avoid any possible malignant diseases.

This article uses NGS and MLPA technology to validate the genomic study and report the first domestic child with OCA2 combined with PWS. OCA2 and PWS are sporadic malignant diseases, and easy to misdiagnose. This case report not only provides clinical insights into OCA2 and PWS diseases, but also preventive clues of complex diseases. For example, PWS combined with Hashimoto's thyroiditis, PWS combined with Metabolic syndrome, PWS combined with Diabetic ketoacidosis, PWS combined with Diabetes, PWS combined with Scoliosis. Besides, this study demonstrates the significance of genetic counseling and genetic testing in reducing the incidence of malign genetic diseases in children. Therefore, in the case of children with unclear diagnosis and suspected ocular and skin albinism and/or PWS, it is necessary to complete the genetic testing as soon as possible to make a clear diagnosis, achieve early diagnosis and treatment, and improve the prognosis of the children. At the same time, genetic counseling can be provided to parents, to prevent the occurrence of disease, to provide a guarantee for the birth of healthy children.

OCA2 combined with PWS due to OCA2 gene missense mutation combined with large fragment deletion of 15q11-13 region was first reported in this study, of which the clinical signs can be subtle and symptoms can be more severe. This case report not only provides clinical insights into OCA2 and PWS diseases, but also the understanding of the clinical phenotype and pathogenesis of OCA2 and PWS was strengthened. Also, this study demonstrates the significance of genetic counseling and genetic testing in reducing the incidence of malign genetic diseases. therefore, early genetic testing is crucial for those patients to yield an accurate diagnosis and initiate aggressive interventions to optimize the outcomes.

Abbreviations

OCA2: Oculocutaneous albinism type 2; PWS: Prader-Willi syndrome ; OCA: Oculocutaneous albinism; MLPA: Multiplex Ligation Dependent Amplification; NGS: Next Generation Sequencing; AS: Angelman syndrome; UPD: Uniparental Disomy; SNRPN protein: Small nuclear ribonucleoprotein associated protein N; snRNPs: small ribonucleoproteins

Declarations

Acknowledgements

Not applicable.

Authors' contributions

GW participated in the study design and writing of the manuscript. DY, MZ and WL participated in clinical data collection. PJ carried out the interpretation of data. XZ participated in data analysis, interpretation of data and writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data and material used or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This report was approved by the Ethics Committee of the Children's Hospital of Soochow University (No.2019LW002). The patients provided written informed consent for participating in this study.

Consent for publication

Written informed consents were obtained from the patients for publication of this manuscript.

Competing interests

The authors declare that they have no competing interests.

References

1. Boycott KM, Vanstone MR., Bulman DE, et al. Rare-disease genetics in the era of next-generation sequencing: discovery to translation. Nat Rev Genet. 2013; 14(10): 681-691.
2. Barness LA. The metabolic and molecular bases of inherited disease, 7th ed. R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle, Vol. 3, McGraw Hill, New York, 1995, pp. 4605. Am J Med Genet. 1996; 66(1): 87-87.
3. Cassidy SB, Schwartz S, Miller JL, et al. Prader-willi syndrome. Genet Med. 2012; 14(1): 10-26.
4. Cheng T, Orlow SJ, Manga P. Loss of Oca2 disrupts the unfolded protein response and increases resistance to endoplasmic reticulum stress in melanocytes. Pigment Cell Melanoma Res. 2013; 26(6): 826-834.
5. Chen K, Manga P, Orlow SJ. Pink-eyed Dilution Protein Controls the Processing of Mol Biol Cell. 2002; 13(6): 1953-1964.
6. Dalay N. Detection of RB1 Gene Copy Number Variations Using a Multiplex Ligation-Dependent Probe Amplification Method. In: *Methods Mol Bio* 2018;1726: 7–18.
7. Delahanty RJ, Zhang Y, BichellTJ, et al. Beyond Epilepsy and Autism: Disruption of GABRB3 Causes Ocular Hypopigmentation. Cell rep. 2016; 17(12): 3115-3124.
8. Duan H, Zheng H, Li H. Mutations and polymorphisms of the P gene associated with oculocutaneous albinism type II. Yi chuan= Hereditas. 2005; 27(26): 984-988.
9. Down JL. ABSTRACT OF THE Lettsomian Lectures ON SOME OF THE MENTAL AFFECTIONS OF CHILDHOOD AND YOUTH. The Lancet. 1887; 129(3306): 62-63.
10. Gardner JM, Nakatsu Y, Gondo Y, et al. The Mouse Pink-Eyed Dilution Gene: Association with Human Prader-Willi and Angelman Science. 1992; 257(5073): 1121–1124.
11. Jones M, Bisht K, Savage SA, et al. The shelterin complex and J Clin Invest. 2016; 126(5): 1621–1629.
12. Ju H, Lee S, Lee J, et al. Necdin modulates osteogenic cell differentiation by regulating Dlx5 and MAGE-D1. Biochem Biophys Res Commun. 2017; 489(2): 109–115.
13. Kamaraj B, Purohit R. Mutational analysis of oculocutaneous albinism: a compact Biomed Res Int. 2014; 2014: 905472.
14. Li Y, GengX, BaoL, et al. A deletion in the Hermansky–Pudlak syndrome 4 (Hps4) gene appears to be responsible for albinism in channel catfish. Mol Genet Genomics. 2017; 292(3): 663–670.
15. Liu C, ZhangX, WangJ, et al. Genetic testing for prader-willi syndrome and angelman syndrome in the clinical practice of guangdong province, Mol Cytogenet. 2019; 12: 7.
16. Mártinez-García M, Montoliu L. Albinism in Europe. J Dermatol. 2013; 40(5): 319–324.
17. Oetting WS, Garrett SS, Brott M, et al. P gene mutations associated with oculocutaneous albinism type II (OCA2). Hum Mutat. 2005; 25(3): 323.
18. Preising MN, Forster H, Tan H, et al. Mutation analysis in a family with oculocutaneous albinism manifesting in the same generation of three Mol Vis. 2007;13: 1851–1855.

19. Rooryck C, Morice-Picard F, Elçioglu NH, et al. Molecular diagnosis of oculocutaneous albinism: new mutations in the OCA1-4 genes and practical Pigment Cell Melanoma Res. 2008;21(5): 583–587.
20. Rodriguez-Jato S, Nicholls RD, Driscoll DJ, et al. Characterization of cis-and trans-acting elements in the imprinted human SNURF-SNRPN locus. Nucleic Acids Res. 2005; 33(15): 4740–4753.
21. Saluja G, Azad SV, Pujari A, et al. Oculocutaneous albinism with iridofundal colo BMJ Case Rep. 2018; 2018: bcr2017223799.
22. Woolf CM. Albinism (OCA2) in Amerindians. Am J Phys Anthropol. 2005; Suppl 41: 118–140.
23. Wang Y, WangZ, ChenM, et al. Mutational Analysis of the TYR and OCA2 Genes in Four Chinese Families with Oculocutaneous Albinism. PLoS One. 2015; 10(4):
24. Zellweger H, Schneider HJ. Syndrome of Hypotonia-Hypomentia-Hypogonadism-Obesity (HHHO) or Prader-Willi Syndrome. Am J Dis Child. 1968; 115(5): 588–598.

Tables

Table 1 Statistics of phenotypes, and genotypes of children with PWS and other diseases reported in China

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Study Participant
gender	Female	Female	male	male	male	Female
age	13 years old	29 years old	9 years old	9 years old	16 years old	6 h
genotype	del15q11	del15q11	del15q11	del15q11, NPHP1 Gene fragment duplication	del15q11	del15q11. 2, OCA2 c.2056G > A
disease	PWS	PWS	PWS	PWS	PWS	PWS
Combined disease	Scoliosis; Nutritional diabetes; Fatty liver; Thyroid dysfunction	Diabetes; Sleep apnea syndrome	Diabetic ketoacidosis; Acute severe pancreatitis; Hypertriglyceridemia	Metabolic syndrome (Hypertension; Hypertensive heart disease; Severe fatty liver; Sleep apnea syndrome)	Hashimoto's thyroiditis	OCA2
Intellectual development	Intellectual development is worse than peers	Faint expression, inferior intelligence and cognitive	Clear consciousness, unclear speech, slow speaking	Backward development in language and intelligence	Growth is a little backward, language development is obviously backward	Lower intelligence
Body type	Obese	Obese	Centripetal obesity	Obese	Centripetal obesity	Trunk obesity
Special face	Full moon face, white complexion, thick black hair, low hairline, no widening of the eye distance	Full moon face, buffalo back, thin upper lip, the skin color of both crotches is red	Moderate dehydration, small jaw, small hands and feet, narrow eyes, almond eyes	The hair is light brown, the eyes are cracked and upturned, the iris is light yellow, and the arch is slightly higher.	No typical PWS special face shape	Because the child is young, it has not been shown yet
Sexual organ development	Labia minora absent, clitoris undeveloped	Breast dysplasia	Short penis	Cryptorchidism	Small penis, small testis	Because the child is young, it has not been shown yet
Limbs	Shorthands, feet and toes, negative bilateral Hoffmann sign, suspected positive bilateral Babinski sign	The hands and feet are short, and the fingers are thin. Several scratches and pigmentation can be seen on the back of both hands, and the lower limbs are denuded.	The extremities are slightly colder	Hands pass through bilaterally, The left foot is turned over, bilateral lower limbs are rotated, flat feet, the physiological reflex can be elicited, pathological signs are negative	The hands and feet are small, and the fingers are slender	Low muscle tone

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Study Participant
other	Scoliosis deformity, chest segment protruding to the right, waist segment protruding to the left, right shoulder 2.5 cm higher than left shoulder, adaman test 2 cm of right razor deformity	Usually emotionally unstable, irritable and irritable, and has the behavior of self-damaging skin, and sometimes feels sleepy at night	Deep breathing, skin elasticity is slightly worse	Emotional agitation, irritability and aggressive behavior, no duplication of nphp1 gene-phenotype	Grumpy, sleep disorders, low vision, diffuse thyroid enlargement	Difficult feeding in infancy, died two months later due to feeding difficulties and pulmonary infection

Figures

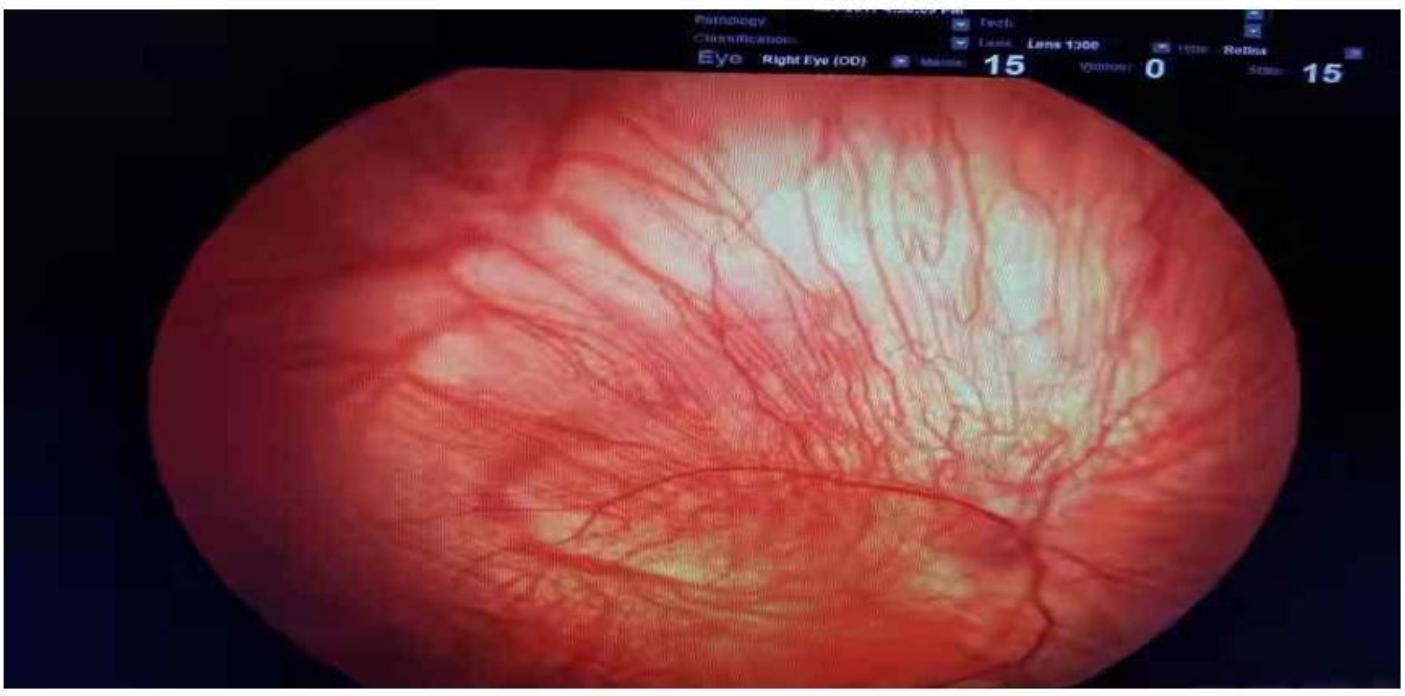

Figure 1

The fundus photo of the right eye shows a clear view of choroidal vasculature due to the hypopigmentation of retinal pigment epithelium, pale retina, foveal hypoplasia, and indistinct optic disc margin.

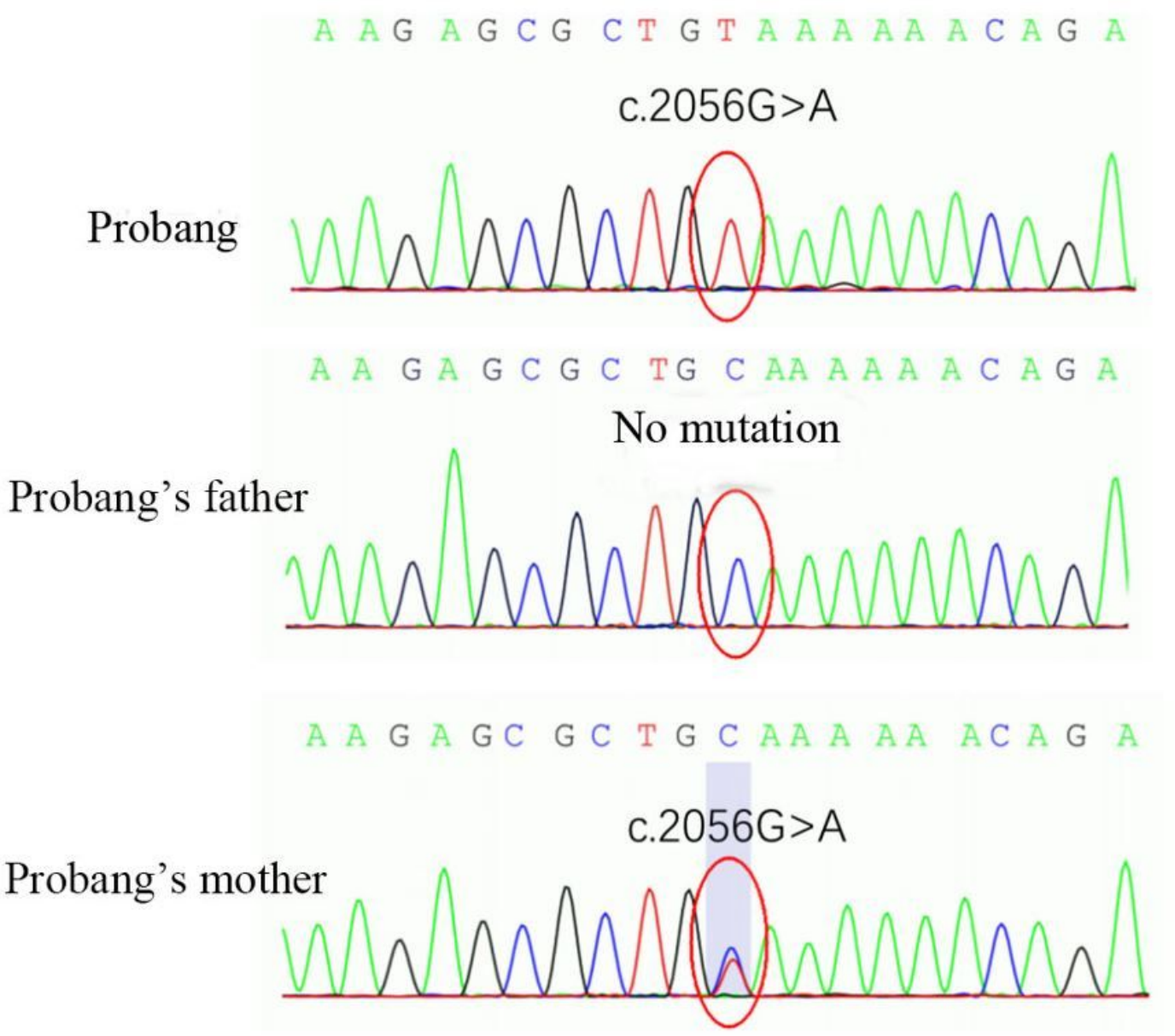

Figure 2

Sanger DNA sequencing revealing the sequence information of the OCA2 gene in children and their parents. The red circle shows the position of c.2056G> A mutation. The child has detected the OCA2 gene c.2056G> A hemizygous mutation, the mother of the child has c.2056G> A heterozygous mutation, the child's father does not carry this Missense mutation.

Figure 3

Sanger DNA sequencing revealing the sequence information of the conservative analysis of children's missense mutations. Chr15: 28171296 at the missense mutation site is highly conserved in the evolution of multiple species.

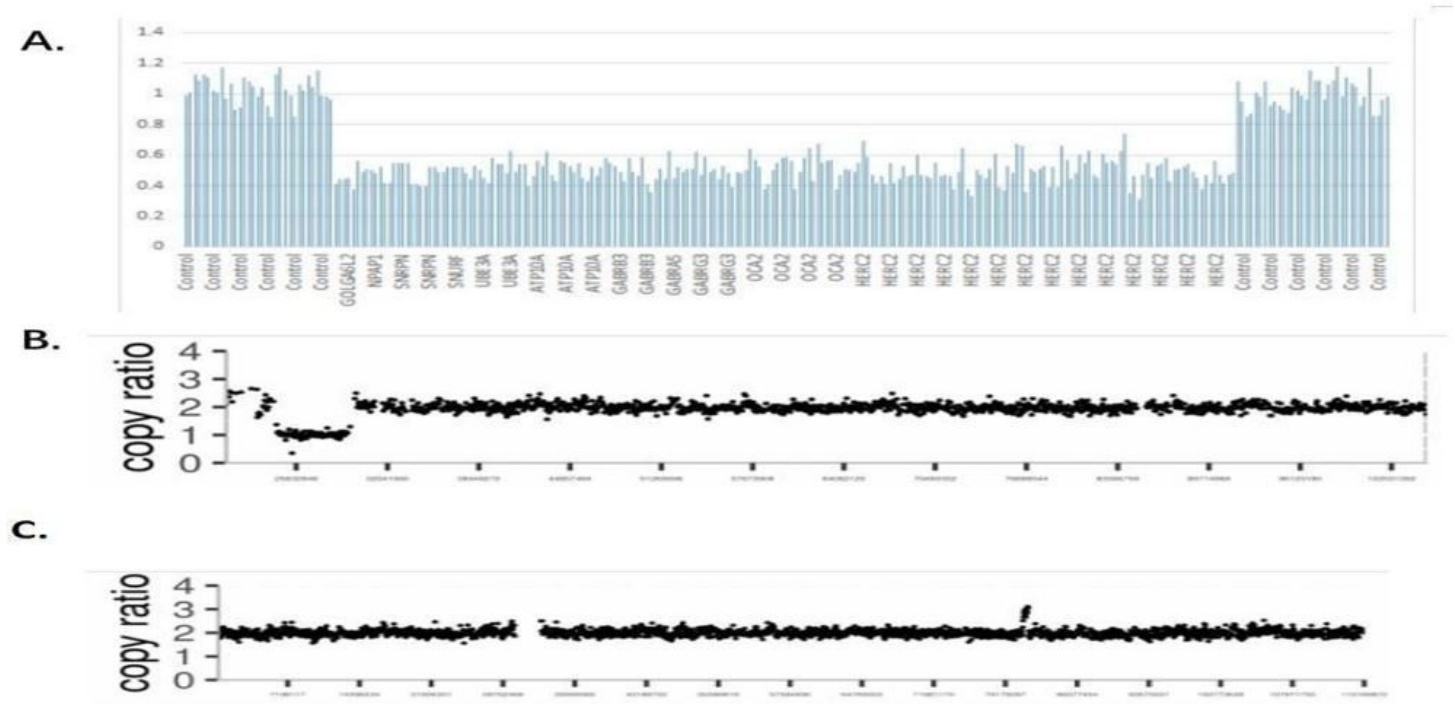

Figure 4

The image showing the patient carrying large fragments missing. A. Extensive results show that there is a suspected paternal heterozygous large fragment deletion in the 15q11-13 region of the child. B. MLPA results confirm that the child carries a deletion variant Del (15q11. 2q13. 1) [GRCh37 / hg19] (23, 378, 392-28, 563, 050) × 1, fragment size: 5.18 Mb. C. Del (15q11. 2q13. 1) [GRCh37/hg19] (23, 378, 392-28, 563, 050)x1, fragment size: 5.18 Mb.

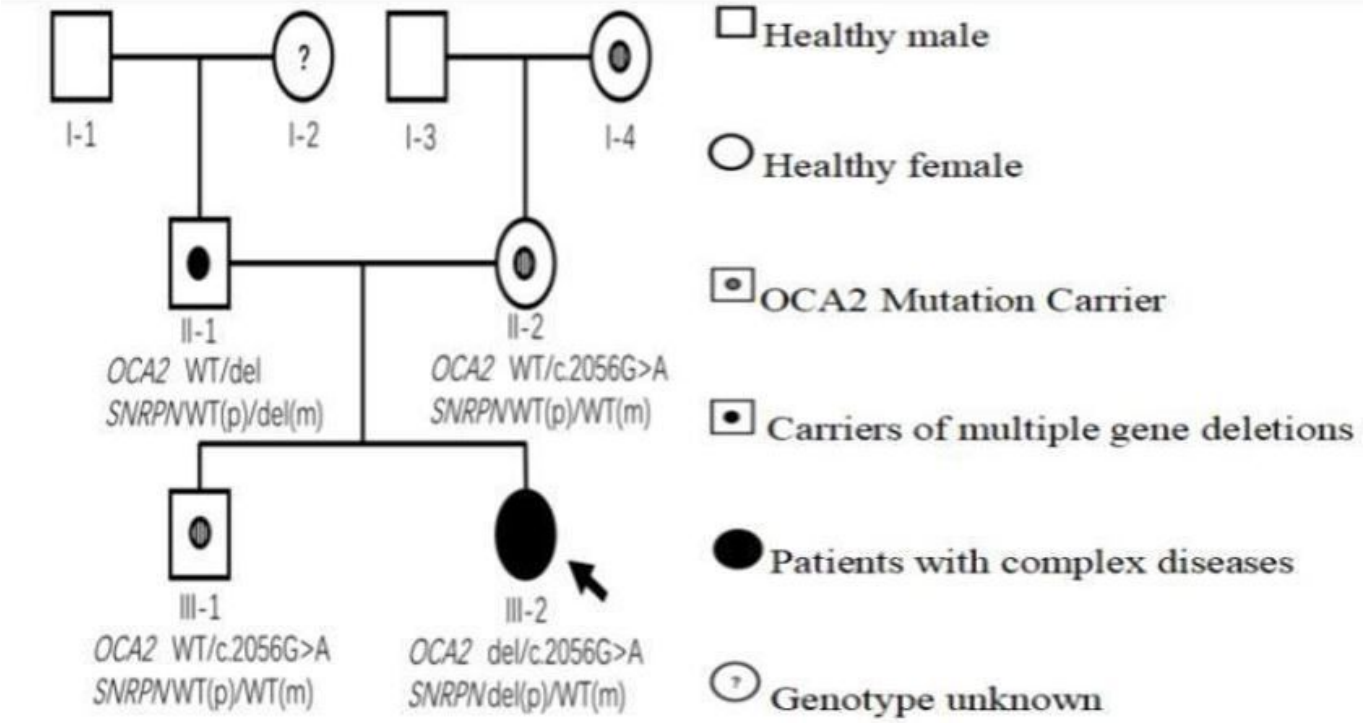

Figure 5

The pedigree analysis showing the family map of the patient. According to the family survey, the father of the proband had growth retardation during childhood. However, no consequences of the growth retardation were observed in adulthood. Moreover, the father of the proband was never diagnosed with PWS. The proband's brother, mother, and grandmother had yellow hairs; Other family members were recorded to be healthy without any genetic defects. Parents were married to distant relatives. With this information, we combined the sequencing results to determine the genotypes of each family member and extrapolated a genetic map.