Causal variants screened by whole exome sequencing in a patient with maternal uniparental isodisomy of chromosome 10 and a complicated phenotype

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Abstract. Uniparental disomy (UPD), which is the abnormal situation in which both copies of a chromosomal pair have been inherited from one parent, may cause clinical abnormalities by affecting genomic imprinting or causing autosomal recessive variation. Whole Exome Sequencing (WES) and chromosomal microarray analysis (CMA) are powerful technologies used to search for underlying causal variants. In the present study, WES was used to screen for candidate causal variants in the genome of a Chinese pediatric patient, who had been shown by CMA to have maternal uniparental isodisomy of chromosome 10. This was associated with numerous severe medical problems, including bilateral deafness, binocular blindness, stunted growth and leukoderma. A total of 13 rare homozygous variants of these genes were identified on chromosome 10. These included a classical splice variant in the *HPS1* gene (c.398+5G>A), which causes Hermansky-Pudlak syndrome type 1 and may explain the patient's ocular and dermal disorders. In addition, six likely pathogenic genes on other chromosomes were found to be associated with the subject's ocular and aural disorders by phenotypic analysis. The results of the present study demonstrated that WES and CMA may be successfully combined in order to identify candidate causal genes. Furthermore, a connection between phenotype and genotype was established in this patient.

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Introduction

Uniparental disomy (UPD), which is the abnormal situation in which both copies of a chromosomal pair have been inherited from one parent (1), may affect either a whole chromosome or only part of a chromosome. The latter is termed segmental UPD and has previously been associated with rearrangements in acrocentric chromosomes that frequently occur in Beckwith-Wiedemann syndrome (2,3). Four mechanisms underlie chromosomal UPD formation: i) Trisomy rescue due to trisomic zygote formation resulting from the failure of two homolog chromosomes to segregate into two daughter cells during parental meiosis; ii) Gamete complementation resulting from fertilization between a nullisomic fertilization and a disomic gamete; iii) Monosomy rescue resulting from mitotic endoduplication in a monosomic zygote; and iv) Postfertilization error during postzygotic mitosis (4,5). There are two types of UDP, which are associated with various mechanisms; the first is uniparental heterodisomy, in which the affected chromosome exhibits two different alleles transmitted from the same parent, and the second is uniparental isodisomy (UPiD), in which the affected individual has two copies of the same allele (4).

Since UPD was initially reported in the 1980s (1), studies employing chromosomal microarray analysis (CMA) and whole exome sequencing (WES) technologies have described UPD for nearly every chromosome in the human genome (6,7). CMA is a first-tier clinical diagnostic test that is used to detect genomic copy number variations (CNVs) in patients with developmental disabilities and congenital anomalies (8). Furthermore, CMA permits researchers to identify UPDs in a straightforward manner (8). WES, which is a type of next-generation sequencing technology, is able to efficiently identify genetic defects in patients with sporadic diseases, as well as providing the mutant information of candidate genes involved in UPD (9). Recently, it has been

demonstrated that WES has the underlying capacity to detect CNVs (10,11). In addition, it may be suitable for detecting UPD (12).

The present study reports the case of a pediatric patient with an undiagnosed and complex medical manifestation who was shown to have UPD at chromosome 10. WES was employed to search for potential causal variants in the patient and to identify connections between candidate genes and the observed phenotypes.

Materials and methods

Patient. A 20-month-old female infant was referred to the Genetics Department at Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine (Shanghai, China) due to multiple severe inborn abnormalities and a severe delay in physiological development after birth (Fig. 1). The patient's mother has had one pregnancy and had delivered a child once, and the child was born at 37 weeks gestation by cesarean delivery due to placental maturation. The patient had an Apgar score (13) of 7'-9' and was comparatively small for her gestational age, with a birth weight of 1,800 g and a length of 40 cm. The patient's parents were well-educated, not biologically related to each other, and were physically and mentally healthy. The parents claimed that they had not been exposed to any toxic materials. The conception had been natural and the course of the pregnancy uneventful. The patient exhibited feeding difficulties, constipation, severe malnutrition and retardation of growth and development after birth.

A physical examination showed the weight and height of the patient to be 4,200 g and 50 cm, respectively. The patient's head circumference was 37 cm and anterior fontanel size was 2x2 cm. The patient had a mild cleft palate, orbital hypertelorism, a long philtrum and blepharophimosis, and was unable to open her right eye. In addition, the patient had dry skin with depigmented spots of various shapes and sizes distributed over her entire body. The patient exhibited no heart murmur, difficultly breathing nor hepatosplenomegaly. She had female-appearing genitalia with a normal urethral opening and a well-proportioned upper and lower body, anus and vaginal opening. Otoacoustic emission, acoustic impedance and brainstem auditory evoked potentials tests demonstrated that the patient had bilateral deafness; corneal light reflex test demonstrated that the patient had binocular blindness. Furthermore, the patient had muscular hypotonia and was unable to raise her head slightly from the prone position. With the exception of crying, the patient was unable to produce any sound.

No unusual results were detected following a laboratory examination involving routine tests of the blood, urine, electrolytes, glucose level and liver, renal and thyroid functions. Neither magnetic resonance imaging (MRI) (Ingenia 1.5T; Philips Healthcare, DA Best, The Netherlands) of the patient's brain, nor screening for inborn metabolic disorders by tandem and gas-chromatography mass spectrometry (micrOTOFI system; Bruker Corporation, Billerica, MA, USA), were able to detect any abnormalities. The study was approved by the ethics committee of Shanghai Children's Medical Center, Shanghai Jiaotong University School of

Figure 1. Abnormalities in the patient. The left picture shows the patient's ophthalmic disorder; her right eye was closed and she was unable to open it. Both the left and right pictures show that the patient has sporadic white patches in her skin across her entire body.

Medicine. Written informed consent was obtained from the child's parents.

CMA. The genomic DNA of the patient was isolated from peripheral blood samples (2 ml) using a QIAamp® DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany). Genomic hybridization was conducted using a CytoScan® HD Array kit (Affymetrix, Inc., Santa Clara, CA, USA), according to the manufacturer's protocol. The array is characterized by >2,600,000 CNV markers, including 750,000 genotype-able single nucleotide polymorphism (SNP) probes and >1,900,000 non-polymorphism probes. Data were visualized and analyzed using the Chromosome Analysis Suite (ChAS 3.1; Affymetrix, Inc.) software package with a minimal cut-off of 20 consecutive markers for CNV calling. All reported CNVs were based on the National Center for Biotechnology Information Human Genome Build 37 (hg19) (http://genome.ucsc.edu/cgi-bin/hgGateway).

WES. DNA (3 μg) from the patient was sheared to create 150-200 bp fragments using a Covarias M220 Ultrasonicator (Covaris, Woburn, MA, USA). The adapter-ligated library was prepared using a SureSelectXT Human All Exon Kit (Agilent Technologies, Santa Clara, CA, USA) as previously described (14), with coding exons and flanking intronic regions enriched. Subsequently, clusters were generated by isothermal bridge amplification using an Illumina cBot system (Illumina, San Diego, CA, USA), and sequencing was performed with an Illumina HiSeq 2000 system.

A quality assessment of base calling and sequence reads was conducted using an HCS 2.2.58 software (Illumina) and an Illumina HiSeq 2000 system including new versions of HiSeq Control Software and Real-Time Analysis. The alignment of sequence reads to a reference human genome (Human 37.3, SNP135; http://hgdownload.soe.ucsc.edu/goldenPath/hg19/snp135Mask/) was performed using NextGENe® software (version 2.4.1; SoftGenetics, LLC., State College, PA, USA). All single nucleotide variants (SNVs) and indels were saved in a VCF file format, and uploaded to the Ingenuity Variant Analysis platform (Qiagen GmbH) for biological analysis and interpretation.
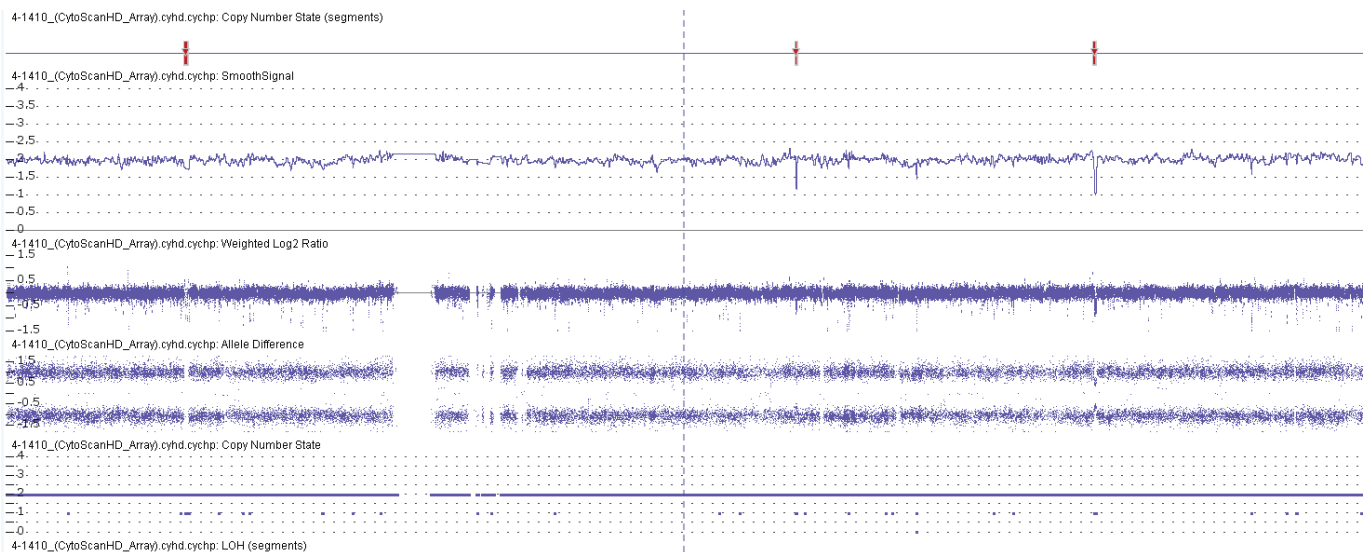
Figure 2. Chromosome microarray analysis results for chromosome 10. Array results showed the copy number state and Log2 ratio of chromosome 10 to be normal. Conversely, the tracks of allele difference lacked the middle track, which is indicative of whole chromosome loss of heterozygosity (LOH). LOH and the absence of copy number variants suggested that the patient had uniparental isodisomy of entire chromosome 10.

In silico analysis. The candidate variants obtained from WES were first screened by the databases of the 1000 Genomes Project (http://www.1000genomes.org/), the National Heart, Lung and Blood Institute Exome Sequencing Project Variant Server (http://evs.gs.washington.edu/EVS/) and the public Complete Genomics (http://www.completegenomics.com/public-data/). Evaluation of the pathogenicity of the candidate variants was performed using the PolyPhen2 online software (http://genetics.bwh.harvard.edu/pph2/).

*Sanger sequencing of the Hermansky-Pudlak syndrome 1 (*HPS1*) gene.* Sanger sequencing was performed as previously described (15). The primers for amplification of the *HPS1* gene (GenBank accession number, NM_000195.3; http://www.ncbi.nlm.nih.gov/genbank/) were designed using the UCSC ExonPrimer online software (http://genome.ucsc.edu/index.html). The primers for exon 5 were as follows: Forward, 5'-GGCATCTTATCA AACCCGCC-3' and reverse, 5'-ACCAACCAGCTAGAT GACCC-3'. The exons and exon-intron boundaries were amplified by polymerase chain reaction (PCR). The reaction mixture for each amplification contained 1X Premix Taq (Ex Taq version 2.0; RR003; Takara Biotechnology Co., Ltd., Dalian, China), 100 ng genomic DNA and 1 pmol forward and reverse primer in a final volume of 25 μl. The reaction was performed in the following PCR conditions: Initial denaturation at 95°C for 5 min, then 19 cycles of 95°C for 30 sec, 65°C for 30 s and 72°C for 45 sec, 14 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 45 sec, and a final elongation step at 72°C for 5 min using a C1000TM Thermal Cycler (Bio-Rad Laboratories, Inc. Hercules, CA,USA). PCR products were separated by 1% agarose gel (Sangon Biotechnology Co., Ltd., Shanghai, China) electrophoresis and purified using a QIAquick Gel Extraction kit (Qiagen GmbH). The purified DNA was sequenced using the ABI3730XL sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with forward and reverse

primers. The results were analyzed using a 3730xl DNA Analyzer (Thermo Fisher Scientific, Inc.). The sequence data was analyzed using the Mutation Surveyor DNA Variant Analysis software (version 4.0.4; SoftGenetics, LLC).

Results

CMA analysis. The CMA analysis using SNP probes suggested that the genome of the patient had a loss of heterozygosity without any CNVs in chromosome 10, which implied that the patient had UPiD of the entire of chromosome 10 (UPiD10) (Fig. 2).

DNA sequencing. The authors of the present study hypothesized that autosomal-recessive variants associated with UPiD10 may have contributed to the clinical manifestations in the patient. Therefore, WES was conducted in order to identify underlying variants. To further characterize chromosome 10, variants in the 1000 Genomes Project, the National Heart, Lung and Blood Institute Exome Sequencing Project Variant Server (http://evs.gs.washington.edu/EVS/) and the public Complete Genomics (http://www.completegenomics.com/public-data/) genomes, were screened for SNPs with a minor allele frequency (MAF) of <3%. The area of analysis included each exon and ~20 bp at exon-intron boundaries. A total of 427 SNVs and 43 indels were shown to meet the filter criteria; these variants covered all chromosomes and the majority were in the heterozygous state (451/470; Table I). For chromosome 10, there were 13 SNVs present in the homozygous state and no SNVs in the heterozygous state, which was consistent with the results of the CMA. Among these, two candidate genes with intron variations were predicted to be silent and not alter protein translation and expression (*PFKFB3*, c.873+13C>T; *MMS19*, c.2776-15G>A).

Among the remaining 11 homozygous variants on chromosome 10 (Table II), the classical splice variant in the *HPS1* gene (c.398+5G>A), which results in the skipping of

Table I. Summary of variants detected by whole exome sequencing (minor allele frequency <0.03).

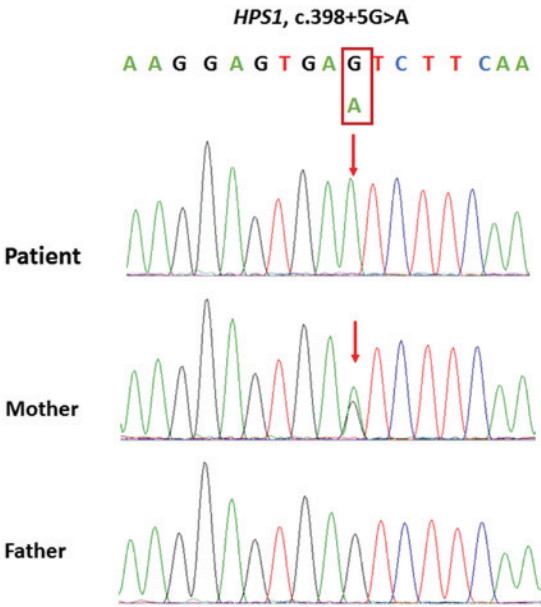| Parameter	Homozygous (Chr. 10)	Heterozygous
Total variants	19 (13)	451
Missense	14 (11)	311
Stop gain	0	9
Frameshift	0	14
Splicing	1 (1)	1
In-frame	0	10
Intron	4 (1)	106

Figure 3. Verification of the c.398+5G>A mutation of the *HPS1* gene by Sanger sequencing. Sequencing of the *HPS1* gene showed the patient to be homozygous for c.398+5G>A, the mother to be heterozygous for the loci, and the father to be homozygous for the wild-type *HPS1* gene.

exon 5 in *HPS1* mRNA (16), was hypothesized to be associated with the ocular and dermal disorders of the patient. An in-depth analysis of the remaining 10 homozygous variants was conducted, and the results did not show any direct association with the clinical manifestations of the patient. Subsequently, the *HPS1* gene in the parents of the patient was evaluated using Sanger sequencing. The sequencing results demonstrated that the patient's mother was heterozygous for c.398+5G>A, whereas the patient's father was wild-type (Fig. 3). These results suggested that the patient has maternal UPiD10.

None of the candidate genes could be associated with the deafness of the patient; thus this feature was used to filter the WES results. All symptom-associated SNVs were filtered using the MAF criterion. In addition, they were predicted by the PolyPhen2 software and compared to the database established by the Department of Laboratory Medicine, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine (containing the WES results of >200 Chinese individuals). An autosomal dominant deafness

causal gene, *MYO1A*, was shown to have a heterozygous mutation (c.1630C>T, p.R544 W), which PolyPhen2 predicted to be the likely cause of deafness in the patient. Furthermore, an analysis using ocular symptoms identified five heterozygous variants and one homozygous variant (Table III), of which the *NR2E3* gene had a heterozygous mutation (c.1127C>T, p.P376L), which may have been associated with the autosomal dominant retinitis pigmentosa and may also have been the cause of the patient's ocular symptoms. The other three candidate genes (*NEB*, *HMX1* and *KCNV2*) were autosomal recessive, indicating that heterozygous mutations were not the cause of the disease.

Discussion

The first reported case of a clinical manifestation attributable to UPD was cystic fibrosis, which was caused by maternal UPD of chromosome 7 (17). Numerous pathogenic chromosome UPDs have since been reported, including those of chromosomes 6, 11, 14, 15 and 20 (4). In the majority of cases, the UPD leads to imprinting disorders (ID), where the UPD event involves genomic imprinting, which alters epigenetic regulation and DNA methylation and histone modifications (18). Angelman syndrome is a well known ID [Online Mendelian Inheritance in Man (OMIM), #105830], which is caused by paternal UPD of chromosome 15, leading to the lack of expression of the maternally inherited *UBE3A* gene (19). With the exception of IDs, UPDs may cause disease if there is a mutation in a recessive gene, where two identical mutant alleles in the proband are inherited from a heterozygous father or mother. The best example of this is cystic fibrosis, in which the underlying molecular mechanism is the C*FTR* gene mutation as a result of UPiD7 (17,20).

The present study reports a case of a pediatric patient with UPiD10 associated with numerous severe medical problems, including bilateral deafness, binocular blindness, stunted growth and leukoderma. To the best of our knowledge, such complicated clinical features have not previously been reported in the literature. CMA was used to diagnose the patient with UPiD10. To date, there are only five cases of UPD10 reported in the literature and all UPD10s have been maternal; however, none of the previously described cases have involved genomic imprinting (21-25). The CMA did not reveal any CNVs on chromosome 10; therefore, the authors of the present study predicted that the etiology of the patient may be caused by rare recessive mutations on chromosome 10. Chromosome 10 of the patient was analyzed by WES, and 11 candidate genes were shown to have homozygous variants. Unlike prior studies of UPD10, in the present study, no single candidate gene was able to fully explain the patient's complex clinical manifestations (26).

The *HPS1* gene encodes a protein that may have a role in the biogenesis of organelles, including melanosomes, platelet dense granules and lysosomes (27). In addition, mutations in *HPS1* lead to Hermansky-Pudlak syndrome type 1 (OMIM, #203300), which is characterized by oculocutaneous albinism, hemorrhagic diathesis, ceroid-lipofuscin accumulation and pulmonary fibrosis (16,28). Thus, this may in part explain the phenotypes associated with the present patient's eyes and skin. A number of the other identified variants, including *ITGA8*, *SEC31B*

Table II. Rare homozygous variants on chromosome 10.

Gene	Position (hg19)	Transcript variant	Translation impact	PolyPhen-2 function prediction	Frequency (1,000 Genome)	Associated Disease (OMIM)	Function
HPS1	10:100195024	c.398+5G>A	Splice site loss		NA	Hermansky-Pudlak syndrome 1	Involved in organelle biogenesis associated with melanosomes, platelet dense granules and lysosomes
ITGA8	10:15726034	c.537C>A	Missense p.S179R	Probably damaging	NA	Renal hypodysplasia	Has an important role in wound-healing and organogenesis
HACD1	10:17659338	c.1A>G	Start loss p.M1V	Benign	0.0004		Regulation of cardiac development and differentiation
SEC31B	10:102275940	c.116C>G	Missense p.T39R	Probably damaging	NA		Unknown
PRDX3	10:120928705	c.701C>T	Missense p.T234I	Probably damaging	0.0040		Regulation of cellular proliferation and differentiation; antioxidant
CACNB2	10:18828169	c.1343G>A	Missense p.G462D	Benign	NA	Brugada syndrome 4	A member of the voltage-gated calcium channel gene
IFIT1	10:91162664	c.632G>A	Missense p.R211H	Benign	0.0008		May inhibit viral replication and translation initiation
HOGA1	10:99358604	c.284G>A	Missense p.R95H	Benign	NA	Primary hyperoxalurea type III	Catalyzes the final step in the metabolic pathway of hydroxyproline, releasing glyoxylate and pyruvate
PAX2	10:102586802	c.1127A>C	Missense p.Q376P	Benign	0.0004	Papillorenal syndrome; renal hypoplasia; focal segmental glomerulosclerosis 7	Regulation of mesenchyme-to-epithelium transition in renal development
WDR11	10:122618246	c.290T>C	Missense p.V97A	Benign	NA	Hypogonadotropic hypogonadism 14	Regulation of cell cycle progression, signal transduction, apoptosis and gene expression
CFAP46	10:134660611	c.6092G>A	Missense p.R2031K		NA		Unknown

OMIM, Online Mendelian Inheritance in Man; NA, not available.

Table III. Probable pathogenic variants associated with the ocular and aural disorders of the patient.

Classification of variants	Gene	Position (hg19)	Transcript variant	Translation impact	Het/ Hom	Mode of inheritance	Associated disease (OMIM)
Ocular genes							
1	*NEB*	2:152506779	c.7342C>T	Missense p.R2448C	Het	Recessive	Nemaline myopathy 2
2	*HMX1*	4:8869775	c.691G>A	Missense p.A231T	Het	Recessive	Oculoauricular syndrome
3	*KCNV2*	9:2718925	c.1186G>A	Missense p.G396R	Het	Recessive	Retinal cone dystrophy 3B
4	*HPS1*	10:100195024	c.398+5G>A	Splice site loss	Hom	Recessive	Hermansky-pudlak syndrome type 1
5	*NR2E3*	15:72109919	c.1127C>T	Missense p.P376L	Het	Dominant or recessive	Enhanced S-cone syndrome; retinitis pigmentosa 37
6	*HPS4*	22:26879985	c.129_142del	Frameshift p.R44fsx10	Het	Recessive	Hermansky-pudlak syndrome type 4
Aural genes							
1	*MOY1A*	12:57432326	c.1630C>T	p.R544W	Het	Dominant	Autosomal dominant deafness

Het, heterozygous; Hom, homozygous; OMIM, Online Mendelian Inheritance in Man.

and *PRDX3,* were predicted to be potentially damaging using PolyPhen2 software; however, they could not be associated with the clinical manifestations of the patient. Whether these genes exacerbated symptom caused by the *HPS1* gene mutation is unclear and requires further study. Direct sequencing of the *HPS1* genes in the parents revealed that the mother had heterozygous loci; thus suggesting that the UPiD10 of the patient was maternal. The authors of the present study hypothesize that UPD10 may always involve a maternal chromosome; however, the underlying mechanism requires further investigation.

In order to improve our understanding of the pathogenesis of the patient, the WES data was filtered using the clinical features, including symptoms of the ears and eyes. Using this strategy, one homozygous variant of the *HPS1* gene, and six heterozygous variants located on other chromosomes, were identified. Among these heterozygous variants, the *MYO1A* gene encodes a member of the myosin superfamily and has a role in actin-based molecular motors (29). Mutations in this gene have previously been associated with autosomal dominant deafness (OMIM, #607841) (29). The *NR2E3* gene encodes a retinal nuclear receptor whose activity is essential to proper rod and cone photoreceptor development and maintenance (30). Defects in this gene are one cause of retinitis pigmentosa 37 (OMIM, #611131), which is characterized by retinal pigment deposits, rod and cone degeneration and loss of vision (31). Various types of retinitis pigmentosa are autosomal dominant (31,32). Therefore, in the present case, the *NR2E3* variant may have a gene dosage effect on the occurrence and development of ocular disorders and may have rendered the illness more complicated.

WES and CMA permit efficient identification of genetic variations. However, they pose significant challenges in terms of data analysis; in particular in the determination of associations between genotypes and complex phenotypes. Despite this, as the use of sequencing technologies and CMA becomes increasingly widespread, the associations between genotypes and phenotypes may become better understood and cases resembling the present report may become more common.

In conclusion, the present study aimed to establish the connection between the patient's phenotype and genotype. The *HPS1* gene provided the clearest explanation of this patient's ocular and dermal disorders, while the *MOY1A* gene may have a role in her deafness and the *NR2E3* gene may have exacerbated her ocular symptoms. At present, little is known regarding the functions of several of the candidate genes (*CFAP46* and *SEC31B*), and their roles in the clinical manifestations of the patient remain unclear.

Acknowledgements

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