Uniparental isodisomy caused autosomal recessive diseases: NGS-based analysis allows the concurrent detection of homogenous variants and copy-neutral loss of heterozygosity

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Abstract

Background: Uniparental disomy (UPD) leading to autosomal recessive (AR) diseases is rare. We found an unusual homozygous state in two nonconsanguineous families, and only one parent in each family was a heterozygote.

Methods: Two patients with homozygosity for pathogenic variants were revealed by whole-exome sequencing (WES), further Sanger sequencing found that only one of the parents was a heterozygote. Initial genotype and copy number variations analysis from WES data of probands involving whole chromosomes 1 and 9 containing these two pathogenic variants were performed, genome-wide single-nucleotide polymorphism (SNP) array analysis was used to confirm these results.

Results: Whole-exome sequencing identified a homozygous c.3423_3424delTG mutation in AGL in patient 1 and a homozygous c.241-1G>C mutation in SURF1 in patient 2. Further parental testing found that only the two patients’ healthy fathers were heterozygous. WES-based copy number and genotype analysis found a copy-neutral loss of heterozygosity (LOH) of whole chromosome 1 in patient 1 and of whole chromosomes 9 and 10 in patient 2. Further genome-wide SNP array and family haplotype analyses confirmed whole paternal uniparental isodisomy (UPiD) 1 in patient 1 and paternal UPiD 9 and maternal UPiD 10 in patient 2. Therefore, UPiD caused AR monogenic glycogen storage disease type-III (GSDIII) in patient 1 and Leigh syndrome in patient 2 through non-Mendelian inheritance of two mutant copies of a gene from each patient’s father.

Conclusion: Our report highlights that a single NGS-based analysis could allow us to find homozygous sequence variants and copy-neutral LOH in such cases. Our report also describes the first case of GSDIII caused by UPiD 1 and Leigh syndrome caused by UPiD 9.

KEYWORDS
AGL, glycogen storage disease type-III, leigh syndrome, SURF1, uniparental isodisomy
1 | INTRODUCTION

Uniparental disomy (UPD) describes both copies of a chromosome pair inherited from only one parent. UPD is classified into uniparental isodisomy (UPiD) and uniparental heterodisomy (UPhD). UPiD refers to the inheritance of two copies of the same chromosome from one parent, while UPhD describes the inheritance of both homologous chromosomes from one parental pair (Liehr, 2010). UPD may not result in adverse effects on individuals; however, UPD can result in clinical conditions by producing either homozygosity for recessive mutations or aberrant patterns of imprinting in humans (Robinson, 2000). Both UPiD and UPhD could result in imprinting disorders, while only UPiD or UPhD could result in autosomal recessive (AR) disorders through the inheritance of deleterious alleles from a carrier parent.

Recently, we encountered two patients with homozygosity for pathogenic variants revealed by whole-exome sequencing (WES), and further parental testing found that only one of the parents was a heterozygote. Initial genotype analysis from WES data of probands revealed a copy-neutral LOH region involving whole chromosomes 1 and 9 containing these two pathogenic variants, which were confirmed by single-nucleotide polymorphism (SNP) array analysis. In a recent study, an approximately 1 Mb segmental copy-neutral LOH region was detected by WES data analysis, which was verified by microarray analysis (Soler-Palacín et al., 2018). WES mainly focuses on the detection of SNVs/indels in rare Mendelian diseases, and copy number variations (CNVs) analysis in the WES bioinformatics pipeline is increasingly common. Several reports using WES data to detect CNVs have been reported (D’Aurizio et al., 2016; Miyatake et al., 2015; Tan et al., 2014). In contrast, the use of WES data for LOH detection is limited (Bis et al., 2017; King et al., 2014; Soler-Palacín et al., 2018), for the SNPs in WES are not pangenomic, and most SNPs with a high allele frequency are located in intronic regions, which limits their use in LOH detection. In this study, a single WES data analysis was initially performed to detect pathogenic variants, CNVs and LOH in two patients with the non-Mendelian inheritance of two mutant copies of a gene from one parent.

2 | MATERIALS AND METHODS

2.1 | Patients

Patient 1 was a Han Chinese girl, and she was the only daughter of a nonconsanguineous couple. The family history was unremarkable. She was first noticed to have hepatomegaly at the age of 4 months. Liver biopsy was performed at 1 year and 6 months of age, and histological examinations showed glycogen accumulation. She came to our clinic at the age of 1 year and 8 months, and her mental development was delayed. Physical examination showed hepatomegaly, short stature, and muscle weakness. She had experienced episodes of hypoglycemia. Her echocardiogram was normal. Her blood biochemistry tests results were listed in Table 1.

Patient 2 was a girl who was the first child of healthy and nonconsanguineous parents. The family history was unremarkable. She was born following 32 weeks of pregnancy. Her birth weight was 880 g (<3rd). She has shown failure to thrive since birth. At 15 months old, psychomotor regression was observed. She showed a mildly delayed developmental profile for her chronological age based on the Gesell Developmental Schedules. The patient’s bone age was 6 months. At 17 months old, aggravation of vomiting was present, and she was admitted to the inpatient department of our hospital for hydrocephalus treatment at 18 months old. An operation was then performed, and an acute deterioration with seizures and vomiting emerged after the operation. Her height, weight, and head circumference at 18 months of age were 70 cm (<3rd), 8 kg (<3rd), and 48 cm (85th-97th), respectively. Laboratory investigation revealed increased lactate (6.5 mmol/L) (normal range 0.7–2.1) and normal glycemia, creatinine kinase, amino acids, and organic acids. Computed tomography of the brain showed hydrocephalus. Her echocardiogram showed an atrial septal defect. Abdomen ultrasound showed normal results.

Informed consent was provided by the parents of both patients for a DNA study and the publication of clinical features. The study protocol was approved by the Ethical Review Board of Xin Hua Hospital (XHEC-D-2014–044).

2.2 | Next-generation sequencing

Genomic DNA was extracted from peripheral whole blood using a QIAamp DNA Blood Mini Kit (Qiagen) following standard procedures. The capture kits used for patient 1 and

| Parameters | Test value | Normal range |
|------------|------------|--------------|
| Creatine phosphokinase | 587 U/L | 26–192 |
| LDH | 605 U/L | 106–211 |
| Aspartate aminotransferase | 439 U/L | 8–38 |
| Alanine aminotransferase | 251 U/L | 0–75 |
| r-GT | 238 U/L | 16–73 |
| Uric acid | 363 µmol/L | 155–357 |
| Fasting blood glucose | 1.05 mmol/L | 3.9–6.1 |
| Triglycerides | 3.38 mmol/L | 0.2–2.31 |
| Cholesterol | 5.09 mmol/L | 3.36–6.46 |
| Lactate | 1.2 mmol/L | 0.7–2.1 |
in patient 1 (Figure 1) and a homozygous SURF1 NM_003172.3:c.241-1G>C mutation in patient 2 (Figure 2). These two mutations were confirmed by Sanger sequencing and showed that both patients’ healthy fathers were heterozygous for the mutation and that their mothers did not carry the mutation. Considering the autosomal recessive inheritance of AGL and SURF1 deficiency, which were not consistent with the homozygous occurrence in two patients. These findings suggested the possibility of maternal chromosome 1 deletion involving the AGL locus or the possibility that the patient 1 inherited two copies of the mutant paternal AGL allele, and for the patient 2, either maternal chromosome 9 bore a deletion involving the SURF1 locus or that the patient inherited two copies of the mutant paternal SURF1 allele.

The patient 1’s WES data showed an LOH region of whole chromosome 1 involving the AGL gene (Figure 1). The patient 2's WES data showed an LOH region of whole chromosome 9 involving the SURF1 gene and LOH of whole chromosome 10 (Figure 2; Figure S1). SNP array and WES-based copy number analyses of the two patients’ genomic DNA yielded normal results, thereby ruling out genomic copy number abnormalities.

A genome-wide SNP array of the family 1 (the patient 1 and her parents) showed that the patient 1 was homozygous for a haplotype present in the father in the whole chromosome 1 region. We identified 50 common SNPs within the LOH region in which the patient 1 had not inherited any allele from her mother (Table S1). Collectively, these results indicate that the homozygous state of the patient 1’s c.3423_3424delITG (p.Glu1142fs*24) mutation was due to paternal isodisomy of whole chromosome 1. A genome-wide SNP array of the family 2 (the patient 2 and her parents) showed paternal isodisomy of whole chromosome 9, and the patient 2 was homozygous for a haplotype present in her father in the whole chromosome 9 region. Specifically, within the LOH region, we identified 30 SNPs in which the patient 2 had not inherited any allele from her mother (Table S2). Collectively, these results indicate that the homozygous state of the patient 2’s c.241-1G>C mutation was due to paternal isodisomy of chromosome 9. In addition, the SNP array of the family 2 showed maternal isodisomy of whole chromosome 10, and the patient 2 was homozygous for a haplotype present in her mother in the whole chromosome 10 region. We identified 31 SNPs within chromosome 10 in which the patient 2 had not inherited any allele from her father. Karyotype analysis of the patient 2 and their parent revealed no chromosomal aberrations involving chromosome 9 and 10.

These two mutations were not present in the 1,000 Genomes Project, gnomAD, EVS or in-house databases, and c.3423_3424delITG mutation in patient 1 had previously been reported in the literature. According to the American College of Medical Genetics (ACMG) guidelines for variant interpretation (Richards et al., 2015), c.3423_3424delITG mutation
could be classified as pathogenic (PVS1+PM2+PP4), and c.241-1G>C mutation could be classified as pathogenic (PVS1+PM2+PP4). The clinical features and identified mutations observed in our two patients were submitted to the AGL and SURF1 variant database (https://databases.lovd.nl/shared/individuals/AGL or SURF1) in the Leiden Open Variant Database (Fokkema et al., 2011).

4 | DISCUSSION

We report an unusual homozygous state in two nonconsanguineous families and determined that only the fathers in these two families were heterozygous for the mutation. Such non-Mendelian inheritance implied the possibility of gross deletion of the mother's allele, nonpaternity, UPD, or a de novo mutation (Tamura et al., 2015). WES and genome-wide SNP array analyses excluded the deletion of the maternal allele, and family haplotype analysis using data from the SNP array confirmed that paternal isodisomy of whole chromosome 1 was present in patient 1 and that complete paternal isodisomy of chromosome 9 and maternal UPiD 10 were present in patient 2. Therefore, UPiD caused autosomal recessive GSDIII in patient 1 and Leigh syndrome in patient 2 through the non-Mendelian inheritance of two mutant copies of a gene from the father.

GSDIII and Leigh syndrome are AR monogenic diseases that are always caused by the inheritance of two mutant alleles from each parent. All reported patients with a molecular diagnosis of these two disorders carry homozygous or

![FIGURE 1](image-url) Whole UPiD 1 of paternal origin containing a homogenous AGL c.3423_3424delTG mutation in patient 1. (a) Family pedigree of patient 1. (b) Sanger sequencing of patient 1 and her parents: a homogenous AGL NM_000028.2: c.3423_3424delTG mutation was identified in the proband, and her father was heterozygous for the mutation. (c) The variant allele frequency plot of the genome (upper panel) and chromosome 1 (lower panel) from the exome sequencing data of patient 1. The x axis shows the position of each SNP site along the chromosome. The y axis represents the variant allele distributions. (d) Copy number and allele peak analyses of the SNP array in patient 1 and her parent. The allele peak analysis of patient 1 showed a 250 Mb LOH region in whole chromosome 1 (with a loss of the middle bands across entire chromosome 1); copy number panels revealed two copies of each gene on chromosome 1. Haplotype analysis using the SNP genotype from the array showed the paternal origin of whole chromosome 1. (e) Family of patient 1 shows the inherited chromosome 1 with the whole isodisomy of paternal origin.
compound heterozygous mutations. This is the first GSDIII patient and the first patient with Leigh syndrome caused by whole UPiD. GSDIII is caused by a deficiency in the glycogen debrancher enzyme and is associated with an accumulation of abnormal glycogen with short outer chains. It is characterized by hepatomegaly, hypoglycemia, and growth retardation. Muscle weakness in patients with IIIa is minimal in childhood but can become more severe in adulthood; some patients develop cardiomyopathy. From the clinical viewpoint, patient 1 presented classical clinical features of GSDIII and no other typical symptoms of GSDIII, suggesting no paternally imprinted genes on chromosome 1 that have a major effect on phenotype. Leigh syndrome is a rare, progressive neurodegenerative mitochondrial disorder of infancy with onset within the first months or years of life and may result in early death. Affected individuals usually show global developmental delay or developmental regression, hypotonia, ataxia, dystonia, ophthalmologic abnormalities, and classic findings on brain imaging. Biochemical studies in patients with Leigh syndrome tend to show increased lactate and abnormalities of mitochondrial oxidative phosphorylation. Clinical features of patient 2 showed typical features of Leigh disease and no other typical symptoms of Leigh syndrome, which also suggests that no paternally imprinted gene on
The incidence of UPD is estimated to be approximately 1:3500 live births (Robinson, 2000; Yamazawa, Ogata, & Ferguson-Smith, 2010) and may not result in adverse effects on the individual if it does not involve chromosomes with imprinted regions. However, UPD could lead to an AR disease through the inheritance of deleterious alleles from a carrier parent. UPD in an AR disease was first reported in a cystic fibrosis patient caused by UPD7 in 1988, which is thought to be a rare mechanistic basis for inherited disease (Spence et al., 1988). Similar cases with segmental or whole-chromosome UPD leading to recessive disorders were subsequently published that involved various chromosomes with different autosomal recessive diseases, in which whole UPD accounted for 60% and segmental UPD accounted for 40% (Niida, Ozaki, Shimizu, Ueno, & Tanaka, 2018). In the reported chromosomes with AR diseases, segmental or whole UPD 1 is the most frequent, while UPD 9 is rare; only two patients with segmental maternal UPD 9, a patient with whole maternal UPD 9 and a patient with complete paternal UPD 9 have been reported, making our patient 2 the second paternal UPD 9.

WES data have revealed the ability of single WES data to identify different types of genomic lesions, including SNVs, LOH, and CNVs. For these two patients described in this report, WES first revealed the disease-causing mutations in the “homozygous” state inherited from one heterozygous parent. No deletion covering the region was detected by CNV analysis from the WES dataset. The LOH region involved in the whole chromosome was further detected by WES-based analysis. Because LOH analysis from a WES dataset does not incur any additional costs, it is a bonus to incorporate LOH analysis in the WES bioinformatics pipeline, especially in cases with non-Mendelian inheritance. The use of next-generation sequencing (NGS) variant frequency data has allowed the detection of somatic LOH in tissue samples, and the results can be verified by microarray analysis (Margraf et al., 2017). A study applied the novel H3M2 algorithm to index patients’ WES data and successfully detected UPD events (Bis et al., 2017). And in another study, a novel method for detecting UPD from trio genotypes from SNP chip or WES data has developed and allowed for detection of pathogenic UPD events (King et al., 2014). These data demonstrate that the LOH region could be detected directly from the NGS data. However, small segmental LOH would be difficult to reveal due to the uneven spread of SNPs in WES. In a recent report, a patient’s WES data identified the LOH region as small as 1.013 Mb in chromosome 4, which caused a homozygous mutation in the proband (Soler-Palacín et al., 2018). More data are required to establish the size limitation of LOH detected by WES.

From these two cases and other similar cases in the literature, it is essential to determine the molecular basis of such diseases for genetic counseling of recurrent risk evaluation. UPD is mainly caused by a meiotic I/II error and/or postzygotic events when associated with trisomy rescue, monosomy rescue, or gamete complementation (Gardner & Sutherland, 2004). Whole UPD is derived from an accidental error in meiosis, and segmental isodisomy is mainly considered the result of postzygotic mitotic recombination after normal fertilization (Niida et al., 2018). The recurrence risk for siblings is far less than 25%, as in general AR diseases. In patient 2 described herein, prenatal diagnosis was performed for her family in the second pregnancy, and the fetus was found to not inherit the mutant allele.

In summary, we reported the first glycogen storage disease type-III (GSD-III) patient with a reported homozygous mutation in AGP caused by whole UPD 1 and the first Leigh syndrome patient with a novel homozygous mutation in SURF1 caused by whole UPD 9 through the non-Mendelian inheritance of two mutant copies of a gene from the father. A comprehensive molecular analysis is necessary to identify UPD-associated AR diseases in which the recurrence risk is low. A single NGS-based analysis could allow us to find a homozygous sequence variant and copy-neutral LOH in such cases.

**CONFLICT OF INTEREST**
None declared.

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SUPPORTING INFORMATION

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