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Whole exome sequencing in a patient with uniparental disomy of chromosome 2 and a complex phenotype

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Abstract

Whole exome sequencing and chromosomal microarrays are two powerful technologies that have transformed the ability of researchers to search for potentially causal variants in human disease. This study combines these tools to search for causal variants in a patient found to have maternal uniparental isodisomy of chromosome 2. This subject has a complex phenotype including skeletal and renal dysplasia, immune deficiencies, growth failure, retinal degeneration, and ovarian insufficiency. Eighteen nonsynonymous, rare homozygous variants were identified on chromosome 2. Additionally, 5 genes with compound heterozygous mutations were detected on other chromosomes that could lead to a disease phenotype independent of the uniparental disomy found in this case. Several candidate genes with potential connection to the phenotype are described but none are definitively proven to be causal. This study highlights the potential for detection of a large number of candidate genes using whole exome sequencing complicating interpretation in both the research and clinical settings. Forums must be created for publication and sharing of detailed phenotypic and genotypic reports to facilitate further biological discoveries and clinical counseling.

Keywords

Bardet-Biedl syndrome; Comparative genomic hybridization; DNA copy number variations; Whole exome sequencing; High-throughput nucleotide sequencing; Uniparental disomy

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Introduction

Recently, the development of next generation DNA sequencing as well as technologies to examine genome wide copy number variation have dramatically changed genetic discovery research (1, 2). Two related technologies—whole exome sequencing and array comparative genomic hybridization (CGH)—are also being utilized by clinicians with increasing regularity and will soon be part of daily practice (3). These tools offer great promise in identifying causal mutations and discovering novel biological pathways. However, the amount of information provided by these types of studies can greatly complicate their interpretation, particularly in the clinical setting. Here, we illustrate the challenges of interpreting exome sequencing results in a patient with an undiagnosed and complex medical presentation who was found to have uniparental disomy (UPD) of chromosome 2.

Case Report

The proband is currently a 21 year old female with multiple medical problems (Fig. 1a). Her parents are nonconsanguineous and are from the Dominican Republic. Severe IUGR was noted at 26 weeks gestation and the patient was born at 35 weeks gestation by Caesarian section with a birth weight of 970 g (−3.6 SDS). Early years were notable for mild global developmental delay; she sat at 12 months, walked at 24 months, had her first words at 18 months, and spoke 2-3 words at 2.5 years. She graduated high school with special education classes and is currently seeking vocational training. She was noted to have a number of dysmorphic features including multiple skeletal anomalies leading to suspicion of a skeletal dysplasia. Her hands are notable for brachydactyly, with a palm length of 8.9 cm (50th centile for a 10 year old) and a middle finger length of 5.5 cm (50th centile for a 6 year old). She also has mild clinodactyly of the 2nd and 5th fingers, some soft tissue syndactyly of the 2nd-4th fingers, and an accessory ossification center at the base of her 2nd metacarpals bilaterally. She has pes planus, widely spaced 1st and 2nd toes, and mild syndactyly of the 2nd and 3rd toes (Fig. 2). She carries a diagnosis of Klippel Feil syndrome with fusion of C2 and C3, shortening of C4 and C5, and partial fusion of C6 and C7 (Fig. 1b). Additionally, she has a dysplastic right kidney.

During childhood, she had recurrent sinusitis, upper respiratory infections, and pneumonias and was diagnosed with hypogammaglobulinemia with low serum levels of all immunoglobulins (IgG, IgM and IgA). She receives intravenous immunoglobulin replacement. She also had frequent herpes stomatitis which led to a diagnosis of depressed natural killer cell toxicity on functional testing.

During evaluation for poor growth, at 12 years old, the subject was found to have growth hormone deficiency with a peak growth hormone level of 1.2 ng/mL to insulin induced hypoglycemia and glucagon stimulation. Concurrently, her peak cortisol level was 12.8 ng/mL leading to a diagnosis of central adrenal insufficiency. A brain MRI showed a low volume pituitary. Growth hormone therapy was given for four years with little overall improvement in her growth velocity. She reached a final adult height of 135 cm (SDS −4.26).

At 12 years and immediately following the initiation of growth hormone treatment, the patient developed diabetes mellitus. She was found to have a normal level of insulin antibodies at 20.8 nU/mL (nl <39 nU/mL), negative insulinoma antigen 2 (IA2) antibodies, but a borderline positive glutamic acid decarboxylase antibody (GAD) at 0.136 units (nl <0.1). She was started on insulin therapy which has been continued to date. The exact etiology of her diabetes is unknown.

At 16 years, she had no breast development or onset of menses, and testing revealed high luteinizing hormone and follicle-stimulating hormone levels of 31.05 IU/L and 75.34 IU/L respectively. Estradiol was low at 11.3 pg/mL consistent with premature ovarian insufficiency leading to hypergonadotrophic hypogonadism. She was started on estrogen replacement therapy resulting in pubertal development.

At 10 years, she was noted to have astigmatism but with excellent corrected vision. At 18 years, the patient began to show signs of retinal degeneration, with attenuated retinal vessels bilaterally and an increased cup to disc ratio. At 19 years, she had inconsistent complaints of visual constriction and poor night vision. She has some symptoms consistent with retinitis pigmentosa (RP), including findings of ring scotoma and attenuated vessels bilaterally, rod and cone degeneration, and a bone spicule in the right eye only. However, some important features of RP such as pallor of the optic nerve and peripheral pigment changes are absent, and no definitive diagnosis has been made. There are no signs of diabetic retinopathy.

The patient is overweight as an adult, with a BMI of 29 kg/m². At 17 years, she presented with hypercholesterolemia with a total fasting cholesterol of 210 mg/dL and LDL of 140 mg/dL. Her cholesterol levels have remained in the borderline range as an adult. At 19 years, she presented with microalbuminuria with urine protein levels ranging from 89.4 – 411.7 mg/g creatinine (nl <20 mg/g), now managed with lisinopril.

Karyotype was normal female 46, XX. There was no DiGeorge/VCFS deletion detected by FISH. Family history is notable for type 2 diabetes and hypercholesterolemia in her mother but is otherwise unremarkable.

Materials and Methods

This study was approved by the institutional review board at Boston Children's Hospital. Written informed consent was obtained from the subject and family members.

The DNA sample was analyzed using Agilent SurePrint G3 custom comparative genomic hybridization and SNP 4×180K microarray (Agilent Technologies, Santa Clara, California), following the manufacturer's instructions. This microarray platform contains 150,000 oligonucleotide probes for the detection of copy number variants (CNVs) and 30,000 probes for the detection of single nucleotide polymorphisms (SNPs). CNV probes were densely populated in genes of interest (detection sensitivity is less than 10 Kb). For the remaining regions of the genome, CNV probe coverage provided detection sensitivity of about 400Kb. SNP probes were evenly distributed across the whole genome; the detection sensitivity for loss of heterozygosity (LOH) is less than 5 Mb. We used Agilent CytoGenomics software package for CNV and LOH calling and visualization. The ADM-2 Quality-Weighted

Interval Score method was used. For CNV detection, a five-consecutive-probe filter was applied.

Whole exome sequencing of the proband was performed at the Broad Institute. Hybrid selection was performed using Agilent's SureSelect Human All Exon Kit v2 (Agilent Technologies, Santa Clara, CA). The sample was sequenced using the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA). We aligned the resulting reads to the hg19 reference genome with BWA(4), applied GATK(5) base quality score recalibration, indel (insertion-deletion) realignment, and performed SNP (single nucleotide polymorphism) and indel discovery and genotyping using variant quality score recalibration (6). Exome sequencing yielded a total of 72,022,554 reads with 93% passing quality filters. Mean target coverage was 74 reads with 79% of target having 30X coverage, 87% having 20X coverage, and 93% having 10X coverage. Variants were annotated for functional effect using SnpEff 2.0.5 (<http://snpeff.sourceforge.net/>). Allele frequency data was obtained from the 1000 Genomes project (February 2012 release) (7) and the NHLBI exome variant server (8).

Confirmation of the 14 out of 18 rare variants on chromosome 2 and 26 rare variants in the 12 potentially compound heterozygous candidates was performed using individual SNP genotyping. SNP genotyping was performed at Boston Children's Hospital using Sequenom iPLEX genotyping (Sequenom, Inc, San Diego, CA, USA). All candidate variants were genotyped in the proband, her parents, and sister as well as 90 HapMap control samples. All passing SNPs had genotype success rates of >90%. Confirmation of the remaining 4 rare variants on chromosome 2 was performed using traditional Sanger sequencing due to genotyping failure or lack of ability to design genotyping primers. The proband, both parents, and her sister were sequenced for these 4 variants. PCR primers and conditions for Sequenom genotyping and Sanger sequencing are available upon request.

Results

CNV microarray with SNP probes did not reveal any copy number variants. However, results suggested that this patient had complete uniparental disomy of chromosome 2 (UPD2) by showing LOH across the entire chromosome (Fig. 3). Data from whole exome sequencing further support the diagnosis of UPD2. We detected a total of 44,813 variants in the patient's exome. Of these, 37.2% of variants showed homozygosity for the allele present. However, on chromosome 2, 95.0% of variants showed homozygosity for the allele present, demonstrating the ability of exome sequencing to identify regions with potential UPD. On further examination of the 5% of variants on chromosome 2 which were called as heterozygous, we noted that the majority were small novel indels of low quality which likely represent sequencing errors.

Given the UPD2, we hypothesized that the subject's phenotype is most likely due to rare homozygous functional variants on chromosome 2. Whole exome sequencing identified 44,813 variants including both single nucleotide variants (39,744) and indels (5,069). Of these total variants, there were 16,678 present in the homozygous state. Table 1 summarizes the breakdown of total variants detected by functional classification and allele count, as well as a summary of variants located on chromosome 2 only. For analysis of candidate variants,

all variants that were present at a minor allele frequency (MAF) greater than or equal to 1% in the 1000 Genomes project, the NHLBI exome variant server, or in 50 HapMap control exomes were excluded leaving 60 rare homozygous variants. Of these, there were 18 nonsynonymous variants in 17 candidate genes, all of which are located on chromosome 2 (Table 2). All 18 variants were validated via confirmatory genotyping or sequencing and were inherited from the proband's mother indicating that she has maternal UPD2. One of these 18 variants is a predicted splice site variant and the others are missense variants. One rare missense variant (MAF = 0.0027), was present in a gene implicated in autosomal recessive retinitis pigmentosa, *FAM161A*, which is a candidate to explain the patient's retinal dystrophy. This variant leads to a L378R substitution in the coded protein which PolyPhen2 (9) predicts is highly likely to be damaging. Although the exact function of this protein remains unknown, truncating mutations in *FAM161A* are a known autosomal recessive cause of retinitis pigmentosa (10), a heterogeneous disease of retinal degeneration characterized by retinal pigment deposits, rod and cone degeneration, and loss of vision (10, 11).

An additional two homozygous variants are located in genes related to kidney structure and function possibly contributing to the patient's history of microalbuminuria and dysplastic kidney. We identified a rare missense variant (MAF = 0.0009) in *NAT8* that leads to a W60S substitution which is predicted by PolyPhen2 to likely be deleterious. The subject also has a rare splice donor site variant (MAF = 0.0014) between exons 21 and 22 in *PLA2R1* in a site that is highly evolutionarily conserved.

Although the presence of UPD2 is the most obvious potential cause of some or all of this patient's clinical picture, some patients with UPD2 have been reported with normal phenotypes, suggesting that some of the findings in this patient could be attributable to mutations on other chromosomes. Therefore, we searched for potential compound heterozygous variants on all chromosomes (there were no rare homozygous nonsynonymous variants detected outside of chromosome 2). We identified 20 genes with two or more rare (MAF < 0.01) nonsynonymous heterozygous variants. Eight of these variant pairs are unlikely to be true compound heterozygotes as the minor allele frequencies are nearly identical, suggesting that these variants are in linkage disequilibrium and most likely present on the same chromosome. We performed confirmatory genotyping and familial segregation of the 26 potential compound heterozygous variants in the remaining 12 genes. All of the variants were validated and were present in the proband in the heterozygous state. The proband's unaffected sister was also a carrier of all the variants from both parents in 7 of these genes ruling them out as candidates. The remaining 5 genes are summarized in Table 3. One of these candidate genes, *NPHP4*, is known to cause a ciliopathy, a category of diseases that share some overlap with our subject's phenotype.

Discussion

We report a subject with a complex phenotype including skeletal and renal dysplasia, immune deficiencies, growth failure, retinal degeneration, and ovarian insufficiency. To our knowledge, this constellation of features has not been previously described in the literature. Chromosomal microarray revealed UPD2 which could lead to her phenotype through three

primary mechanisms: imprinting, homozygous copy number variants, or unmasking of rare pathogenic sequence variants. There are 19 cases of UPD2 reported in the literature (12-30). Importantly, cases of both maternal and paternal UPD2 have been reported in individuals with normal phenotypes, suggesting that imprinting is an unlikely mechanism by which UPD is causing this patient's phenotype (15, 22). Array CGH did not reveal any CNVs on chromosome 2, excluding the potential role of structural variation, to the degree that can be detected by the array (>10 Kb), in explaining the patient's phenotype. Finally, UPD can lead to homozygosity of rare recessive mutations on the involved chromosome (31). Many cases of UPD2 resulting in the unmasking of rare recessive mutations are reported in the literature, including cases of Crigler-Najjar syndrome, Donnai-Barrow syndrome, severe congenital hypothyroidism, Harlequin ichthyosis, infantile-onset ascending spastic paralysis, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency, and mitochondrial DNA depletion syndrome (13, 16, 18, 20, 21, 24). None of these subjects resemble the phenotype found in our proband, or any of its features, with the exception of one individual with retinal dystrophy believed to be caused by a recessive mutation in *MERTK* (27). Our subject did not have a rare variant in this gene, or any genes identified as candidate genes in prior studies of UPD2. We therefore searched for rare recessive mutations in other genes on chromosome 2.

Exome sequencing highlighted 18 rare nonsynonymous homozygous variants in 17 genes, all of which were on chromosome 2 (Table 2). Several candidate genes stood out in our analysis as potentially causal. Our subject has retinal degeneration with some features of retinitis pigmentosa although does not have a definitive diagnosis, and one potential explanation for this phenotype is the variant found in *FAM161A*. Four of the five known pathogenic mutations in this gene are found in exon 3, as is the mutation in our patient. Therefore, it is possible that this variant explains this one aspect of her phenotype. However, all of the known mutations are truncating or frameshift mutations, and it is unclear that our patient's missense variant is truly pathogenic as opposed to an incidental finding of our exome sequencing approach.

Additionally, rare variants were identified in two genes with potential functional effects in the kidney, *NAT8* and *PLA2R1*, which could explain our patient's unilateral dysplastic kidney as well as early onset microalbuminuria. *NAT8* encodes a probable N-acetyltransferase highly expressed in the kidney and liver. It is hypothesized to play a role in the development and maintenance of the structure and function of these tissues, but its biological role is not fully understood (32). *PLA2R1* encodes for a receptor of phospholipase A2 with high levels of expression in the kidney (33). Binding of the ligand leads to activation of MAPK signaling and induces cell proliferation (34). However, it is unclear by what mechanism a defect in *PLA2R1* could cause the phenotype observed in this patient, and mouse knockouts for *PLA2R1* show no overt abnormalities (35). Again, these variants could be pathogenic, but could also be incidental findings.

Finally, the subject has some features consistent with a ciliopathy, a disease category which is associated with defects in proteins that play a role in the structure and function of cilia. Ciliary dysfunction can lead to a number of clinical syndromes with overlapping features, including Meckel-Gruber syndrome, Bardet-Biedl syndrome, Joubert syndrome, nephronophthisis, Senior-Loken syndrome, and Jeune asphyxiating thoracic dystrophy (36).

In particular, the phenotype of our patient resembles Bardet-Biedl syndrome (BBS), which has characteristic features of rod and cone dystrophy, postaxial polydactyly, renal anomalies, obesity (with complications of type II diabetes, hypertension, and hypercholesterolemia), learning disabilities, and hypogonadism (37). While certain aspects of her phenotype are not encompassed by BBS, such as her hypogammaglobulinemia and growth hormone deficiency, it seems plausible that such a diagnosis could at least partially explain the abnormalities we observed.

We found 3 rare mutations (Table 3) in one ciliary gene, *NPHP4*, which is implicated in two ciliopathies, Senior-Loken syndrome and nephronophthisis (38). Phasing indicates that one of these three variants was inherited paternally, and her sister is not a carrier. The mother and sister are both carriers of the other two variants. Thus, it is plausible that a combination of the paternal variant with the maternal variants contributed to the phenotype observed in the patient. Additionally, we found that the subject has a rare homozygous mutation in *ARL6IP6* (Table 2), a gene on chromosome 2 which encodes a membrane protein with no known function. However, this protein was identified because of its interaction with *ARL6*, a causative gene in BBS (37, 39). Ciliopathies like BBS have been proposed to be oligogenic, with evidence for modifying alleles contributing to penetrance and variability in clinical outcome (36, 40, 41). Thus, it could be the case that this mutation in *ARL6IP6*, in combination with the mutations in *NPHP4* is contributing to a BBS-like phenotype.

Array CGH and whole exome sequencing are powerful research tools which are revolutionizing researchers' ability to identify candidate genes in disease phenotypes. These tools are increasingly being utilized in the clinical setting, leading to difficulty in the clinical interpretation of complex results. We present a highly informative case in which array CGH and whole exome sequencing were combined to search for causal genes in a complex patient found to have UPD2. The many variants of unknown significance in this case highlight the difficulty that clinicians will experience as genetic technologies gain more widespread use in everyday practice. The large number of candidate genes identified in this study is in part due to the loss of heterozygosity as a consequence of isodisomy. However, in addition to the 18 rare, homozygous variants on chromosome 2, we also identified 5 genes with similarly rare potential compound heterozygous mutations that could lead to clinical effects independent from the patient's UPD. It is also possible that her phenotype was caused by a *de novo* mutation. Determining this would involve whole exome sequencing of both of her parents, which was beyond the scope of the current study.

In contrast to prior studies of UPD2, we were not able to identify a single candidate gene that could explain the subject's complete, complex phenotypic picture. We highlight a number of potentially causal genes including *FAM161A*, a known cause of retinitis pigmentosa, which could explain this patient's retinal degeneration. Additionally, *NAT8* and *PLA2R1* may play a role in her renal dysplasia and microalbuminuria. Finally, many of her features are consistent with a ciliopathy, such as BBS, and she has a number of rare nonsynonymous variants in BBS-related genes. However, we also found rare mutations in many genes that are unlikely to be involved in her phenotype, such as *TTN*, which encodes for a large component of the sarcomere, and *OR4D1*, an olfactory receptor. We additionally failed to locate a likely cause of certain aspects of her phenotype, such as her immune

deficiencies and her growth hormone deficiency. It is possible that these features of her phenotype are caused by one of the variants we detected, where the causal biology is not yet obvious. Alternatively, these features could be due to differences that are not detected by whole exome sequencing, such as variants in non-coding regulatory regions or epigenetic changes. In summary, we present a number of candidate genes which could explain aspects of her phenotype, yet, we have not definitively proven that any of these are causal.

The high rates of rare variant identification by whole exome sequencing and microarray analysis will make it difficult to distinguish the causal variants from background rare but non-causal variants for any single case. We believe that it is imperative that researchers and clinicians publish similarly complex cases with detailed phenotypic and genotypic data, even when a causal gene has not yet been established, to permit the identification of causal genes that are implicated in multiple cases with phenotypic overlap. Such reports of genotype-phenotype correlations will empower future biological discoveries as well as assist clinical geneticists and genetic counselors in the interpretation of exome sequencing and array CGH results.

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References

1. Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet.* 2011; 12:745–755. [PubMed: 21946919]
2. Bejjani BA, Shaffer LG. Clinical utility of contemporary molecular cytogenetics. *Annu Rev Genomics Hum Genet.* 2008; 9:71–86. [PubMed: 18949852]
3. Need AC, Shashi V, Hitomi Y, et al. Clinical application of exome sequencing in undiagnosed genetic conditions. *J Med Genet.* 2012; 49:353–361. [PubMed: 22581936]
4. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009; 25:1754–1760. [PubMed: 19451168]
5. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010; 20:1297–1303. [PubMed: 20644199]
6. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet.* 2011; 43:491–498. [PubMed: 21478889]
7. A map of human genome variation from population-scale sequencing. *Nature.* 2010; 467:1061–1073. [PubMed: 20981092]
8. National Heart, Lung, and Blood Institute. Exome variant server [homepage on the internet]. Seattle, WA: c2012 [cited 2012 Feb 16]. Available from <http://evs.gs.washington.edu/EVS/>
9. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* 2010; 7:248–249. [PubMed: 20354512]
10. Bandah-Rozenfeld D, Mizrahi-Meissonnier L, Farhy C, et al. Homozygosity mapping reveals null mutations in FAM161A as a cause of autosomal-recessive retinitis pigmentosa. *Am J Hum Genet.* 2010; 87:382–391. [PubMed: 20705279]

11. Langmann T, Di Gioia SA, Rau I, et al. Nonsense mutations in FAM161A cause RP28-associated recessive retinitis pigmentosa. *Am J Hum Genet.* 2010; 87:376–381. [PubMed: 20705278]
12. Albrecht B, Mergenthaler S, Eggermann K, et al. Uniparental isodisomy for paternal 2p and maternal 2q in a phenotypically normal female with two isochromosomes, i(2p) and i(2q). *J Med Genet.* 2001; 38:214. [PubMed: 11303520]
13. Bakker B, Bikker H, Hennekam RC, et al. Maternal isodisomy for chromosome 2p causing severe congenital hypothyroidism. *J Clin Endocrinol Metab.* 2001; 86:1164–1168. [PubMed: 11238503]
14. Baskin B, Geraghty M, Ray PN. Paternal isodisomy of chromosome 2 as a cause of long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. *Am J Med Genet A.* 152A:1808–1811. [PubMed: 20583174]
15. Bernasconi F, Karaguzel A, Celep F, et al. Normal phenotype with maternal isodisomy in a female with two isochromosomes: i(2p) and i(2q). *Am J Hum Genet.* 1996; 59:1114–1118. [PubMed: 8900241]
16. Castiglia D, Castori M, Pisaneschi E, et al. Trisomic rescue causing reduction to homozygosity for a novel ABCA12 mutation in harlequin ichthyosis. *Clin Genet.* 2009; 76:392–397. [PubMed: 19664001]
17. Chevalier-Porst F, Rolland MO, Cochat P, et al. Maternal isodisomy of the telomeric end of chromosome 2 is responsible for a case of primary hyperoxaluria type 1. *Am J Med Genet A.* 2005; 132A:80–83. [PubMed: 15580638]
18. Douglas GV, Wiszniewska J, Lipson MH, et al. Detection of uniparental isodisomy in autosomal recessive mitochondrial DNA depletion syndrome by high-density SNP array analysis. *J Hum Genet.* 2011; 56:834–839. [PubMed: 22011815]
19. Heide E, Heide KG, Rodewald A. Maternal uniparental disomy (UPD) for chromosome 2 discovered by exclusion of paternity. *Am J Med Genet.* 2000; 92:260–263. [PubMed: 10842292]
20. Herzfeld T, Wolf N, Winter P, et al. Maternal uniparental heterodisomy with partial isodisomy of a chromosome 2 carrying a splice acceptor site mutation (IVS9-2A>T) in ALS2 causes infantile-onset ascending spastic paralysis (IAHSP). *Neurogenetics.* 2009; 10:59–64. [PubMed: 18810511]
21. Kantarci S, Ragge NK, Thomas NS, et al. Donnai-Barrow syndrome (DBS/FOAR) in a child with a homozygous LRP2 mutation due to complete chromosome 2 paternal isodisomy. *Am J Med Genet A.* 2008; 146A:1842–1847. [PubMed: 18553518]
22. Keller MC, McRae AF, McGaughan JM, et al. Non-pathological paternal isodisomy of chromosome 2 detected from a genome-wide SNP scan. *Am J Med Genet A.* 2009; 149A:1823–1826. [PubMed: 19610117]
23. Lopez-Garrido MP, Campos-Mollo E, Harto MA, et al. Primary congenital glaucoma caused by the homozygous F261L CYP1B1 mutation and paternal isodisomy of chromosome 2. *Clin Genet.* 2009; 76:552–557. [PubMed: 19807744]
24. Petit FM, Gajdos V, Parisot F, et al. Paternal isodisomy for chromosome 2 as the cause of Crigler-Najjar type I syndrome. *Eur J Hum Genet.* 2005; 13:278–282. [PubMed: 15586176]
25. Spiekerkoetter U, Eeds A, Yue Z, et al. Uniparental disomy of chromosome 2 resulting in lethal trifunctional protein deficiency due to homozygous alpha-subunit mutations. *Hum Mutat.* 2002; 20:447–451. [PubMed: 12442268]
26. Talseth-Palmer BA, Bowden NA, Meldrum C, et al. A 1q44 deletion, paternal UPD of chromosome 2 and a deletion due to a complex translocation detected in children with abnormal phenotypes using new SNP array technology. *Cytogenet Genome Res.* 2009; 124:94–101. [PubMed: 19372674]
27. Thompson DA, McHenry CL, Li Y, et al. Retinal dystrophy due to paternal isodisomy for chromosome 1 or chromosome 2, with homoallelism for mutations in RPE65 or MERTK, respectively. *Am J Hum Genet.* 2002; 70:224–229. [PubMed: 11727200]
28. Wolstenholme J, White I, Sturgiss S, et al. Maternal uniparental heterodisomy for chromosome 2: detection through 'atypical' maternal AFP/hCG levels, with an update on a previous case. *Prenat Diagn.* 2001; 21:813–817. [PubMed: 11746120]
29. Vilchis F, Mendez JP, Canto P, et al. Identification of missense mutations in the SRD5A2 gene from patients with steroid 5alpha-reductase 2 deficiency. *Clin Endocrinol (Oxf).* 2000; 52:383–387. [PubMed: 10718838]

30. Stratakis CA, Taymans SE, Schteingart D, et al. Segmental uniparental isodisomy (UPD) for 2p16 without clinical symptoms: implications for UPD and other genetic studies of chromosome 2. *J Med Genet.* 2001; 38:106–109. [PubMed: 11288708]
31. Yamazawa K, Ogata T, Ferguson-Smith AC. Uniparental disomy and human disease: an overview. *Am J Med Genet C Semin Med Genet.* 2010; 154C:329–334. [PubMed: 20803655]
32. Ozaki K, Fujiwara T, Nakamura Y, et al. Isolation and mapping of a novel human kidney- and liver-specific gene homologous to the bacterial acetyltransferases. *J Hum Genet.* 1998; 43:255–258. [PubMed: 9852678]
33. Ancian P, Lambeau G, Mattei MG, et al. The human 180-kDa receptor for secretory phospholipases A2. Molecular cloning, identification of a secreted soluble form, expression, and chromosomal localization. *J Biol Chem.* 1995; 270:8963–8970. [PubMed: 7721806]
34. Granata F, Petraroli A, Boilard E, et al. Activation of cytokine production by secreted phospholipase A2 in human lung macrophages expressing the M-type receptor. *J Immunol.* 2005; 174:464–474. [PubMed: 15611272]
35. Hanasaki K, Yokota Y, Ishizaki J, et al. Resistance to endotoxic shock in phospholipase A2 receptor-deficient mice. *J Biol Chem.* 1997; 272:32792–32797. [PubMed: 9407054]
36. Waters AM, Beales PL. Ciliopathies: an expanding disease spectrum. *Pediatr Nephrol.* 2011; 26:1039–1056. [PubMed: 21210154]
37. Forsythe E, Beales PL. Bardet-Biedl syndrome. *Eur J Hum Genet.* 2012
38. Fillastre JP, Guenel J, Riberi P, et al. Senior-Loken syndrome (nephronophthisis and tapeto-retinal degeneration): a study of 8 cases from 5 families. *Clin Nephrol.* 1976; 5:14–19. [PubMed: 1248184]
39. Zhang Q, Nishimura D, Seo S, et al. Bardet-Biedl syndrome 3 (Bbs3) knockout mouse model reveals common BBS-associated phenotypes and Bbs3 unique phenotypes. *Proc Natl Acad Sci U S A.* 2011; 108:20678–20683. [PubMed: 22139371]
40. Vinogradov AV. [Differences in ways of development of myocardial infarct with severe and mild clinical course]. *Kardiologiya.* 1990; 30:5–8. [PubMed: 2273737]
41. Schaefer E, Zaloszyk A, Lauer J, et al. Mutations in SDCCAG8/NPHP10 Cause Bardet-Biedl Syndrome and Are Associated with Penetrant Renal Disease and Absent Polydactyly. *Mol Syndromol.* 2011; 1:273–281. [PubMed: 22190896]

Figure 1. Cervical abnormalities

(a) Photo of the patient at age 21. (b) Radiographic image of the cervical spine showing fusion of C2 and C3, shortening of the vertebral bodies of C4 and C5, and partial fusion of C6 and C7.

Figure 2. Upper and lower digits

Upper panel shows images of the dorsal and palmar surfaces as well as a radiographic image of the left hand. Left hand is notable for brachydactyly and clinodactyly, as well as some soft tissue syndactyly of the 2nd to 4th digits. Lower panel shows left dorsal foot and radiographic images of both feet. The patient has pes planus, widely spaced 1st and 2nd toes, and mild syndactyly of the 2nd and 3rd toes.

Figure 3. Microarray results for chromosomes 1 and 2

Array results for chromosome 1 are shown for comparison. There are no abnormalities on chromosome 1. For each chromosome, the upper panel displays the CNV scatter plot showing that no CNV was detected on either chromosome (black dots indicate Cy5/Cy3 ratio between 0.25 and -0.25 ; blue dots indicate Cy5/Cy3 ratio > 0.25 and red dots indicate Cy5/Cy3 ratio < -0.25). The bottom panel displays the zygosity plot. In the zygosity plot, the top and bottom tracks indicate homozygous status while the middle track indicates heterozygous status. In chromosome 1, there are many dots distributed across the heterozygous as well as homozygous tracks. However, on chromosome 2, the paucity of heterozygous SNPs (i.e. lack of dots in the middle track) across the whole chromosome in this sample indicates the whole chromosome loss of heterozygosity. The absence of loss of heterozygosity in the rest of the genome and the copy neutral status of the chromosome 2 indicates the UPD status of chromosome 2 in this subject.

Table 1

Summary of variants detected through exome sequencing.

All Variants			
	Whole Exome		Chromosome 2
Total Variants	44813		2101
% Homozygous	37.2%		95.0%
<i>Nonsynonymous SNP</i>	8980		422
<i>Splice Site</i>	127		
<i>Frameshift</i>	214		6
<i>In-Frame Coding Indel</i>	328		12
<i>Start Gained</i>	142		7
<i>Start Lost</i>	25		2
<i>Nonsense</i>	59		0
<i>Readthrough</i>	14		0
<i>Synonymous SNP</i>	10625		481
<i>Intron</i>	14711		713
<i>Other Non-Coding</i>	9588		455

Minor Allele Frequency < 0.01			
	Whole Exome		1 Chromosome 2
	Homozygous	Heterozygous	Homozygous
Total Variants	60	1647	54
<i>Nonsynonymous SNP</i>	17	468	17
<i>Splice Site</i>	1	3	1
<i>Frameshift</i>	0	9	0
<i>In-Frame Coding Indel</i>	0	12	0
<i>Start Gained</i>	0	5	0
<i>Start Lost</i>	0	2	0
<i>Nonsense</i>	0	11	0
<i>Readthrough</i>	0	0	0
<i>Synonymous SNP</i>	12	406	9
<i>Intron</i>	21	421	19
<i>Other Non-Coding</i>	9	310	8

Table 2

Rare homozygous variants.

Gene	Position (hg19)	Ref	Alt	Frequency (1000 Genomes)	Missense	PolyPhen2 Prediction	Associated Disease (OMIM)	Mouse Knockout Phenotype (MGI)	Function (Uniprot)
CAD	2:27445809	G	A	N/A	R238H	0.209			Pyrimidine biosynthesis, cell proliferation
SULT6B1	2:37406661	G	C	0.0018	P157A	0.995			Sulfotransferase involved in thyroxine metabolism
SMEK2	2:55795486	A	T	0.0005	F593L	0.494			Regulatory subunit of serine/threonine protein phosphatase 4 (PP4) involved in hepatic glucose metabolism
FAM161A	2:62067006	A	C	0.0027	L378R	0.985	Retinitis pigmentosa 28		Unknown, expressed in the retina
NAT8	2:73868577	C	G	0.0009	W60S	0.992			Unknown, may play a role in the development and maintenance of normal kidney and liver structure
YSK4 (variant transcript)	2:135740813	T	C	N/A	Q1121R	0.573			Unknown, serine/threonine protein kinase
DARS	2:136673814	C	T	0.0005	G363E	0.347			Aminoacyl-tRNA synthetase
NEB	2:152522801	G	A	N/A	R1612C	0.999	Nemaline myopathy, type 2	Stunted growth, muscle weakness, postnatal death	Large protein component of the sarcomere
ARL6IP6	2:153575472	C	T	0.0009	L112F	0.004			Unknown, interacts with ARL6
PLA2R1	2:160803929	C	T	0.0014	splice site	N/A		Viable and fertile with no overt abnormalities	Receptor for phospholipase A2, plays a role in MAPK-induced cell proliferation
TTN	2:179399863	G	A	N/A	R32186C	0.999	Cardiomyopathy, muscular dystrophy	Cardiac and skeletal defects, growth retardation, muscle weakness, prenatal death	Large protein component of the sarcomere
COL5A2	2:179638834	C	T	0.0032	R2354H	1.000			
	2:189916110	C	G	0.0018	R956P	0.155	Ehlers-Danlos syndrome, type 1	Reduced and abnormal bone growth, fragile skin, perinatal lethality	Collagen V component
SDPR	2:192711244	G	C	0.0009	C136W	0.981			Phospholipid binding protein, PKC substrate

Gene	Position (hg19)	Ref	Alt	Frequency (1000 Genomes)	Missense	PolyPhen2 Prediction	Associated Disease (OMIM)	Mouse Knockout Phenotype (MGI)	Function (Uniprot)
SLC39A10	2:196545348	C	A	0.0032	H194Q	0.010			Unknown, possible zinc influx transporter
HECW2	2:197208381	G	A	0.0032	P134L	0.001			E3 ubiquitin-protein ligase that ubiquitinates and stabilizes TP73
NBEAL1	2:204009459	A	T	0.0027	Y1633F	0.997			Unknown, has structural similarity to neurobeachin
PSMD1	2:231948431	G	A	<0.0001	R559K	0.103			Regulatory subunit of the proteasome

Table 3

Potential rare compound heterozygous variants.

Gene	Position (hg19)	Ref	Alt	Frequency (1000 Genomes)	Missense	PolyPhen2 Prediction	Carriers	Associated Disease (OMIM)	Mouse Knockout Phenotype (MGI)	Function
NPHP4	1:5924483	T	C	0.0023	H1304R	0.006	F	Nephronophthisis type 4, Senior-Loken syndrome type 1	Photoreceptor degeneration, male infertility	Involved in the organization of apical junctions in kidney cells
	1:5965750	G	C	0.0032	Q569E	0.604	M,S			
SLC6A20	1:6027365	C	T	0.0027	A171T	0.512	M,S			
	3:45804541	C	A	N/A	A443S	0.428	M,S	Hyperglycinuria, iminoglycinuria (digenic)		Transporter of small hydrophilic molecules, in kidney and intestine
	3:45821529	G	T	N/A	A109D	0.428	F			
ADAMTS12	5:33624365	C	T	0.0032	R705K	0.000	F		increased tumor vascularization and invasion	Metalloproteinase that degrades cartilage oligomeric matrix protein
	5:33881583	GCT	G	N/A	N/A	N/A	M			Negatively modulates TGF-beta signaling in keratinocytes
CD109	6:74440187	A	G	N/A	I133V	0.929	F			
	6:74495175	T	C	0.0014	F771S	0.971	M			Olfactory receptor
OR4D1	17:56232873	A	G	0.0032	Y120C	0.999	F,S			
	17:56232977	C	T	0.0041	H1554	1.000	M			