Case Report

Genome sequencing identifies a rare case of moderate Zellweger spectrum disorder caused by a PEX3 defect: Case report and literature review

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A B S T R A C T

Defects in PEX3 are associated with a severe neonatal-lethal form of Zellweger spectrum disorder. We report two moderately affected siblings whose clinical and biochemical phenotypes expand the reported spectrum of PEX3-related disease. Genome sequencing of an adolescent male with progressive movement disorder, spasticity and neurodegeneration, and previous non-diagnostic plasma very-long chain fatty acid analysis, revealed a homozygous likely pathogenic missense variant in PEX3 [c.991G > A; p.(Gly331Arg)]. A younger sibling with significant motor decline since the age of three years was also subsequently found to be homozygous for the familial PEX3 variant. A comprehensive review of the scientific literature identified three additional families with non-lethal infantile- or childhood-onset PEX3-related disease, which together with this clinical report illustrate the potential for highly variable disease severity. Our findings demonstrate the diagnostic utility of genome-wide sequencing for identifying clinically and biochemically heterogeneous inherited metabolic disorders such as the peroxisome biogenesis disorders.

1. Introduction

Peroxisome biogenesis disorders (PBDs; MIM 601539) are caused by variants in genes involved in peroxisomal assembly and function [1]. PBDs include Zellweger spectrum disorders (ZSDs) and rhizomelic chondrodysplasia punctata (RCDP) types 1 [2]. Classification of peroxisome biogenesis disorders in the Zellweger spectrum (PBD-ZSD), ranging from severe (ZS), intermediate (NALD), and mild (IRD) phenotypes, respectively. Individual clinical pictures are along a spectrum of disease severity and often do not fit into the original assigned categories. The extreme variability in disease manifestation ranging from onset of profound neurologic symptoms in newborns to progressive degenerative disease in adults presents practical challenges in disease diagnosis and medical management [2].

There are currently 13 peroxin genes associated with ZSDs [2]. Of these, PEX3, PEX16, and PEX19 encode proteins involved in peroxisomal membrane protein (PMP) biogenesis [3]. Specifically, PEX3 is involved in PMP import and targeting [4,5]. The first three reported cases of PEX3 defect presented with a severe form of ZSD that resulted in early lethality [4,6–9]. While clinical heterogeneity was noted, all three patients shared dysmorphic facial features and severe neurologic findings in addition to clearly abnormal plasma and/or fibroblast very-long chain fatty acids (VLCFA) levels.

PEX3 defects are very rare [10]. A limited number of case reports have described patients with mild to moderate clinical presentations of ZSD who carried pathogenic variants in PEX3, suggesting there may be a broader clinical spectrum for PEX3-related disease [10–12]. Here we report two siblings with a clinically and biochemically nonspecific progressive neurological disorder of initially unclear etiology. Genome sequencing (GS) identified a homozygous likely pathogenic PEX3 variant in an adolescent male with progressive movement disorder, spasticity, and neurodegeneration.

Abbreviations: ES, exome sequencing; gnomAD, Genome Aggregate Database; GS, genome sequencing; IRD, infantile Refsum disease; NALD, neonatal adrenoleukodystrophy; MAF, minor allele frequency; PBD, peroxisome biogenesis disorder; PMP, peroxisomal membrane protein; RCDP, rhizomelic chondrodysplasia punctata; VLCFA, very-long chain fatty acids; X-ALD, X-linked adrenoleukodystrophy; ZSD, Zellweger spectrum disorder; ZS, Zellweger syndrome

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variant, confirming a diagnosis of ZSD. In addition to broadening the clinical spectrum of this rare disease, we use this case to demonstrate the diagnostic utility of GS as a first-line test for identifying clinically and biochemically heterogeneous inherited metabolic disorders.

2. Case report

The siblings were born to consanguineous parents. They were born at term by vaginal delivery with no complications after uneventful pregnancies. Neither had marked neonatal hypotonia nor dysmorphic features. The family reported no history of genetic, neurologic, or kidney diseases.

PI, a 17-year-old male, had normal development until approximately 18 months of age, when he presented with fever associated with ataxia and ophthalmoparesis. He had progressive developmental regression, ataxia, dysphagia and spasticity and by 13 years of age, had complete loss of ambulation and severe speech impairment. At age 16, he was admitted to the hospital with acute kidney injury, secondary to urinary tract obstruction. Neurologic examination revealed horizontal and vertical nystagmus, abnormal smooth and saccadic eye movements, tremor, dystonia and spastic quadriplegia with multiple joint contractures, hyperreflexia and bilateral clonus. Ultrasound showed horseshoe kidneys with nephrocalcinosis and small non-obstructive stones. Imaged liver was normal in size and echotexture. Brain and C-T-L spine MRI at age’s 2-years-11-months and 16-years showed diffuse progressive myelin and white matter loss with atrophy of brain and spinal cord (Fig. 1). Chromosomal microarray showed normal 46, XY results. Metabolic investigations (ammonia, lactate, venous blood gas, creatinine, electrolytes, liver enzymes, acylcarnitine profile, plasma amino acids, CDG, aryl-sulfatase and urine sulfatides, beta-galactosidase, galactocerebrosidase, total hexosaminidase and HEX-B, urine organic acids, homocysteine, copper, ceruloplasmin, free and total carnitine, and B12) were all normal. VLCFA analysis showed mild non diagnostic isolated elevation of C26:0 with minimal elevation of ratios: C24:C22 1.009 (0.543–0.941 umol/L); C26:C22 0.028 (0.005–0.017 umol/L); C26:1 0.757 (0.247–1.095 umol/L); C26:0 1.090 (0.269–0.923 umol/L). A repeat analysis showed moderately elevated C26:C22 ratio but other indices were normal: C24:C22 0.918 (0.543–0.941 umol/L); C26:C22 0.025 (0.005–0.017 umol/L); C26:1 0.634 (0.247–1.095 umol/L); C26:0 0.878 (0.269–0.923 umol/L). Again, this result was reported as non-diagnostic, however it was noted that adrenomyeloneuropathy or X-linked adrenoleukodystrophy could not be ruled out. As imaging studies (Fig. 1) and disease presentation in both male and female paediatric patients were not consistent with ABCD1 mutation, GS was offered to this patient.

Sagittal T1W image (a) in Patient 1 at nearly 3 years of age demonstrates a normal size optic chiasm (small arrow), corpus callosum (arrow), brainstem and cerebellar vermis (long arrow). Sagittal T1W image (f) in Patient 2 at 12 years of age demonstrates loss of volume in all 3 structures. By 16 years of age in Patient 1 (k), the chiasm, corpus callosum (small arrow) and vermis (arrow) have significantly atrophied and there is enlargement of the basal cisterns. Early axial T2W image (b) in Patient 1 shows poor myelin maturation of the cerebellum (arrow) and increased T2 signal (short arrow) in the pontine tegmentum. Similar tegmental signal (g) is present in Patient 2 with the additional feature of cerebellar hemisphere atrophy (arrow). T2W axial image (l) in Patient 1 at 16 years shows further loss of pontine volume (black arrow) and marked enlargement of the interfoliate sulci (arrow).
Early axial T2W image (c) in Patient 1 shows abnormally increased signal (arrow) surrounding the myelin stripe of the posterior limb of internal capsule. Over time in Patient 2 at 12 years (h) and Patient 1 at 16 years (m) abnormal signal replaces the myelin of the posterior (arrows) and anterior limbs of the internal capsule, there is diffuse loss of myelin signal and progressive enlargement of the ventricles and sulci. Normal T1 myelin signal (arrow) is present in Patient 1 on initial MRI (d), but is lost (arrows) by 12 years in Patient 2 (i) and 16 years in Patient 1 (m). FLAIR (e) shows abnormal signal in the posterior limb of internal capsule and periatrial white matter (arrow) in Patient 1. Abnormal signal extends to involve all white matter in Patient 2 (j) (arrow). Diffuse abnormal white matter signal and significant progression in central (arrow) and peripheral volume loss is seen by 16 years of age in Patient 1 image (o).

PIL reached appropriate milestones until age 3 years, when she developed weakness in her legs and has subsequently experienced significant motor decline. This patient's brain MRI showed abnormal myelination at age 5 per her family's report. No records were available to confirm this information. At 12 years of age, she underwent additional MRI imaging showing diffusely abnormal white matter signal. On examination at age 11, the patient was alert and appropriate. She presented with dysarthria, dystonia and dysmetria. She had normal tone in upper limbs and scissoring posture with spasticity in lower limbs. She had contractures in both her ankles and knees and atrophy in thighs and calf muscles. She had multiple small café au lait spots (≤0.5 cm), pectus carinatum, and scoliosis. She used ankle and foot orthoses (AFOs) and was able to walk short distances with a walker.

PBDs are extremely heterogeneous and it is difficult to delineate a clear clinical picture from which to base a reliable differential diagnosis. As the genotypic and phenotypic spectrum of inherited metabolic conditions expands, biochemical testing may not be sufficient for accurate diagnosis for heterogeneous inherited metabolic disorders. It is possible that PBDs are being underdiagnosed in less severely affected patients. While the value of biochemical studies should not be ignored, now with the availability of advanced molecular tools, comprehensive genetic testing should be integrated into the diagnostic system for PBDs. In Japan, a diagnostic system incorporating exome sequencing (ES) has led to an increase in the diagnosis of PBDs in patients with neurological diseases and atypical peroxisomal parameters [18].

In cases where biochemical studies show inconclusive results, GS is a time-efficient alternative to conventional biochemical and genetic testing strategies. It allows for the potential to detect all types of variants including structural and copy number variants as well as variants in regions not covered by ES (e.g., variants in non-coding regions and pseudogenes).

Our patients' diagnostic journey was long and complex. Despite having received an array of tests and medical assessments in several countries, the patient had not received an accurate diagnosis for

### Table 1

|          | P1  | P2  | Normal Controls | X-ALD hemizygote | X-ALD Heterozygote | ZSD   |
|----------|-----|-----|-----------------|------------------|-------------------|-------|
| Phytanic | 0.420 | 0.410 | < 3.00 μg/ml | 1.3 ± 0.45 | 0.68 ± 0.29 | 3.93 ± 1.50 |
| Pristanic | 0.040 | 0.050 | < 0.3 μg/ml | 0.34 ± 0.16 | 0.23 ± 0.10 | 4.08 ± 2.30 |
| C26:0 Hexacosanoic | 0.780 | 0.690 | 0.23 ± 0.09 | 1.71 ± 0.23 | 1.30 ± 0.19 | 2.07 ± 0.28 |
| C24:1 | 0.620 | 0.590 | 0.18 ± 0.09 | 1.31 ± 0.01 | 1.07 ± 0.03 | 1.50 ± 0.16 |
| C24/C22 | 1.165 | 1.117 | 0.84 ± 0.10 | 1.71 ± 0.23 | 1.30 ± 0.19 | 2.07 ± 0.28 |
| C26/C22 | 0.042 | 0.030 | 0.01 ± 0.004 | 0.07 ± 0.03 | 0.04 ± 0.02 | 0.50 ± 0.16 |

P1: Patient 1; P2: Patient 2; X-ALD: X-linked Adrenoleukodystrophy; ZSD: Zellweger syndrome; Mean ± 1SD

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### Table 2
Summary of clinical and imaging phenotypes our and published mild to moderately affected patients with PEX3 mutations.

| Reference | This report | 10 | 11 | 12 | P1 | P2 |
|-----------|-------------|----|----|----|----|----|
| Sex       | M           | M  | M  | M  | F  | M  |
| Ethnicity | Jordanian   | M  | M  | M  | F  | M  |
| Consanguinity | Yes | No | No | Yes | F  | M  |
| Age at dx (yr) | 17 | 11 | 9  | 32 | 26 | 22 |
| Clinical Phenotype | | | | | | |
| Onset     | 18 months   | 3 yr | 8 months | 1 yr 8 months | 5 yr | “never been able to walk” |
| Initial presentation | Fever associated with ataxia and ophthalmoplegia | Weakness in legs | Psychomotor retardation, axial and peripheral muscular hypertonia, nephrocalcinosis | Unable to walk independently, spastic quadriplegia | Unable to walk, medical information from childhood sparse | Medical information from childhood sparse |
| Dysmorphic features | − | − | − | − | − | − |
| Neurological findings | Ataxia, spasticity, dysphagia | Dystonia, dysmetria | Severe spastic paraparesis, brisk reflexes with axial hypertonia, needed motor assistance using a wheelchair by age 4 | Spastic quadriplegia, | Spastic tetraparesis, dysphagia | Spastic tetraparesis, dysphagia |
| Skeletal findings | scoliosis, pectus carinatum | scoliosis, pectus carinatum | − | − | − | − |
| Developmental delay / Intellectual disability | + | + | + | + | + | + |
| Speech impairment / Dysarthria | + | + | NA | + | + | + |
| Ophthalmological findings | Ophthalmoplegia, oculomotor apraxia, horizontal nystagmus | Visual fields normal extraocular movements were intact with interpreted pursuit. | Bilateral cataracts at age 4, nystagmus | Pallor of optic disc, no nystagmus | Nystagmus, visual impairment with severe myopia and papillary pallor | Visual impairment with hyperopia |
| Hearing impairment | NA | − | − | − | − | − |
| Neurogenic bladder | + | − | + | − | + | − |
| Seizures | − | − | + | − | − | − |
| Hepatomegaly | − | NA | + | − | − | − |
| Diagnostic Investigations | MRI | MRI reported to show hypomyelination of brain and spine at age 16 | MRI performed at 26 months, 3 and 4 yr were normal | Frontal lobe atrophy at age 23 | Marked atrophy with general atrophy, especially of the cerebellum, diffuse bilateral white matter hyperintensities on T2 and FLAIR, hypointense lesions in the basal ganglia and corticospinal tract bilaterally on T2-weighted images, radiological features consistent with leukodystrophy |
| Plasma VLCFA | Mildly elevated | Mildly elevated | Mildly elevated | Marked increase | NA | NA |
| C24:0/C22:0 | Mildly elevated | Mildly elevated | Mildly elevated | Marked increase | NA | NA |
| C26:0/C22:0 | Mildly elevated | Mildly elevated | Mildly elevated | NA | NA | NA |
| Fibroblast C26:0 | NA | NA | NA | NA | NA | NA |
| Karyotype analysis | 46 XY normal | 46 XY normal | 46 XY normal | 46 XY normal | 46 XY normal | 46 XY normal |
| Method of mutation detection | GS | Targeted Sanger Sequencing | Sequence analysis of PEX genes | Sequence analysis of PEX genes | ES | ES |
| Mutation | c.991G > A (p.Gly331Arg), homozygous | c.991G > A (p.Gly331Arg), homozygous | c.898C > T (p.Arg300*), homozygous | c.1039G > T (p.Arg347Tyr), homozygous | c.206-1G > T, homozygous | c.206-1G > T, homozygous |

Abbreviations: "−" = absent, "+" = present, yr = year, NA = not available or not reported, M = male, F = female, ES = exome sequencing, GS = genome sequencing, VLCFA = very-long chain fatty acids.
15 years. GS allowed for a hypothesis-free approach to evaluation of both candidate genes and variants types in the search for a diagnosis for our patient.

Timely and accurate diagnosis can not only change the management of the patient, but it also leads to subsequent testing and identification of at-risk family members. In our patient's family, an unaffected sibling will have the option to get carrier testing for future family planning.

GS has demonstrated superiority over conventional genetic testing strategies such as microarrays and next generation sequencing (NGS)-based multigene panels [19,20]. A recent study comparing the analytic and diagnostic performance of GS and ES demonstrated that GS has higher analytic performance than ES [21]. Additionally, GS has the ability to detect structural and copy number variants as well as variants in regions not covered by ES (e.g., variants in non-coding regions and pseudogenes). Thus, the diagnostic yield of GS is expected to increase as our ability to interpret the clinical significance of noncoding and structural variants improve [21]. A repeat VLCFA done for both patients after receiving the GS results showed levels were consistent with a defect in peroxisomal fatty oxidation supporting pathogenicity of the PEX3 c.991G > A variant.

In order to prevent significant diagnostic delays and its subsequent impact on the quality of care these patients receive, GS should be considered a first-line diagnostic test for patients with progressive developmental regression, non-specific VLCFA or peroxisomal pathology. Thus, the diagnosticyield of GS is expected to increase as our ability to interpret the clinical significance of noncoding and structural variants improve [21]. A repeat VLCFA done for both patients after receiving the GS results showed levels were consistent with a defect in peroxisomal fatty oxidation supporting pathogenicity of the PEX3 c.991G > A variant.

PBDs are often difficult to diagnose due to their rarity and clinical and genetic heterogeneity. Timely diagnosis through the use of GS will allow families to receive appropriate care including but not limited to effective intervention for presenting symptoms, monitoring of disease progression, and genetic counselling.

4. Conclusion

This report illustrates the variable expression and severity of PEX3-related disease. Our findings demonstrate the diagnostic utility of GS for identifying clinically and biochemically heterogeneous inherited metabolic disorders.

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Author contributions

Conception, manuscript, preparation and revision of intellectual content: WL, GC, MI.

Acquisition of biochemical, genetic, and clinical data: GC, MI, SB, SW, CM, HG.

Review and critique, and final approval of the manuscript: WL, GC, MI, SB, SW, CM, HG.

Ethical approval

This case study was approved by the Research Ethics Board at SickKids as part of the Genome Clinic research (REB# 1000037726). Informed written consent was obtained from the patient's parents for the Genome Clinic research and specifically for publication of this case report.

Declaraiton of Competing Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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