



## A homozygous mutation in *PEX16* identified by whole-exome sequencing ending a diagnostic odyssey



Carlos A. Bacino<sup>a,c</sup>, Yu-Hsin Chao<sup>a</sup>, Elaine Seto<sup>b,c</sup>, Tim Lotze<sup>b,c</sup>, Fan Xia<sup>a</sup>, Richard O. Jones<sup>d</sup>, Ann Moser<sup>d</sup>, Michael F. Wangler<sup>a,c,\*</sup>

<sup>a</sup> Department of Molecular and Human Genetics, BCM, Houston, TX, 77030, United States

<sup>b</sup> Department of Pediatrics, Division of Pediatric Neurology and Developmental Neuroscience, BCM, Houston, TX, United States

<sup>c</sup> Texas Children's Hospital, Houston, TX, United States

<sup>d</sup> Kennedy Krieger Institute, Baltimore MD, United States

### ARTICLE INFO

#### Article history:

Received 8 July 2015

Received in revised form 3 September 2015

Accepted 3 September 2015

Available online 30 September 2015

#### Keywords:

Functional genomics

Peroxisomal biogenesis

*PEX16*

Ataxia

### ABSTRACT

We present a patient with a unique neurological phenotype with a progressive neurodegenerative. An 18-year diagnostic odyssey for the patient ended when exome sequencing identified a homozygous *PEX16* mutation suggesting an atypical peroxisomal biogenesis disorder (PBD). Interestingly, the patient's peroxisomal biochemical abnormalities were subtle, such that plasma very-long-chain fatty acids initially failed to provide a diagnosis. This case suggests that next-generation sequencing may be diagnostic in some atypical peroxisomal biogenesis disorders.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### 1. Introduction

Genomic sequencing is a successful strategy for the diagnosis of rare inherited disorders [5,17,29]. In addition to gene discovery, sequencing can identify mutations in known disease genes, particularly when the phenotype is unusual or atypical compared to previously reported cases. In the case of metabolic disorders, sequencing can reveal underlying defects previously undetected by biochemical studies, such as Argininemia [18] and mitochondrial DNA depletion disorders [12].

Peroxisomal biogenesis disorders (PBDs) are a group of disorders with global peroxisomal defects due to mutations in the *PEX* genes [25]. Peroxisomes are ubiquitous organelles responsible for the oxidation of very-long-chain fatty acids (VLCFAs) and branched chain fatty acids, synthesis of plasmalogens, and detoxification of free-radicals and glyoxylate [25, 27]. PBDs have a spectrum of severity with Zellweger syndrome, the most severe form, leading to early-onset seizures, polymicrogyria, hepatic dysfunction, bone abnormalities, and early death. Milder phenotypic categories such as Neonatal Adrenoleukodystrophy (NALD) and Infantile Refsum disease (IRD) have been associated with less severe biochemical defects [4,8] but are generally still characterized by severe impairment in infancy. Recently, atypical patients with onset after infancy and primary ataxia mimicking spinocerebellar ataxia have been reported particularly

in association with mutations in *PEX2* [15,22], *PEX10* [20] and *PEX16* [3]. These patients have very mild abnormalities in very-long-chain fatty acid metabolism and plasmalogen biosynthesis.

Here we clinically identify an individual with an ataxia phenotype, which remained undiagnosed for many years. Our patient was ultimately diagnosed by whole exome sequencing (WES), which revealed homozygous *PEX16* mutations. This individual exhibits either barely detectable or normal results in every peroxisomal biochemical assay. These findings suggest a role for next-generation sequencing in the diagnostic workup of atypical variant PBD.

### 2. Materials and methods

#### 2.1. Ethics statement

Informed consent for the research and for publication was obtained prior to participation for the subject who was recruited under an Institutional Review Board approved protocol at Baylor College of Medicine.

#### 2.2. Peroxisomal biochemical studies

Plasma samples and cultured fibroblast from a skin biopsy were used for peroxisomal biochemical analysis. Plasma pipercolic acid was measured by electron capture negative ion mass fragmentography [10]. Very-long-chain fatty acid levels and total lipid fatty acid profile were measured as described [11,16]. The plasmalogen assay was

\* Corresponding author at: Department of Molecular and Human Genetics, BCM, Houston, TX, 77030, United States.

E-mail address: [michael.wangler@bcm.edu](mailto:michael.wangler@bcm.edu) (M.F. Wangler).

performed using C14 radioactivity incorporation and H3 counts to measure microsomal plasmalogen steps [21]. Fibroblast oxidation assays were performed using radioactive substrates to assay enzyme activity [19,26]. Measurement of C26:0-lyso-PC was performed as described [7] and bile acid quantization was performed by tandem mass spectrometry [9]. Catalase distribution in cultured cells was performed and quantified (% soluble catalase) [14,28].

### 2.3. Whole-exome capture, sequencing and data analysis

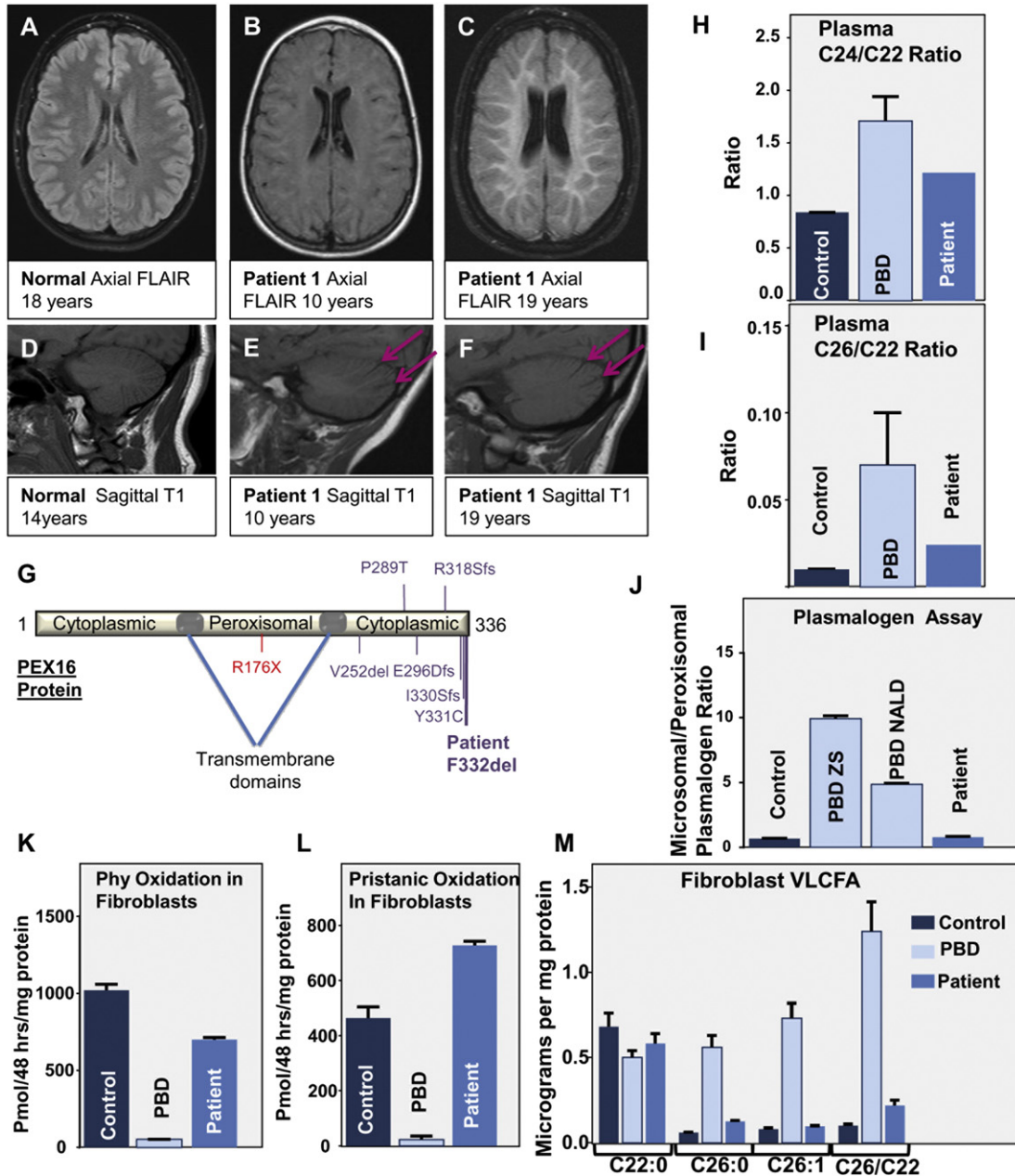
The patient underwent WES through the Whole Genome Laboratory (<https://www.bcm.edu/research/medical-genetics-labs/index.cfm?PMID=21319>) using methods described [13]. Produced sequence

reads were aligned to the GRCh37 (hg19) human genome reference assembly using the HGSC Mercury analysis pipeline (<http://www.tinyurl.com/HGSC-Mercury/>). Variants were determined and called using the Atlas2 [2] suite to produce a variant call file (VCF [1]). High-quality variants were annotated using an in-house developed suite of annotation tools [24].

### 3. Results

#### 3.1. Case report: an unusual clinical presentation of ataxia

A 22 year old female presented at age 3 years with ataxia. An initial MRI was normal. She underwent biochemical and genetic testing



**Fig. 1.** Summary of radiographic, molecular and biochemical analysis. (A) Normal Axial FLAIR sequence in a teenage patient (B–C) Axial FLAIR images for the patient showing a progressive leukodystrophy. (D) Sagittal MRI of a control patient showing a normal cerebellum with normal folia. (E–F) Sagittal MRI of the patient showing abnormal space (purple arrows) between the cerebellar folia, indicating a cerebellar degeneration. (G) Schematic of the PEX16 protein showing the location of important domains as well as reported mutations. A mutation associated with Zellweger syndrome (R176X) is shown in red. Purple mutations are those associated with atypical variant PBD (H) Plasma C24/C22 ratio at age 22 years (Z score 3.75). (I) Plasma C26/C22 at age 23 years (Z-score 3.5). (K–L) Phytanic and Pristanic acid oxidation in fibroblasts showing normal results for the patient. (M) Fibroblast VLCFA oxidation showing a subtle defect most evident in the C26/C22 ratio (Z-score 5.0).

including very long chain fatty acids in plasma which initially showed an amount of C26:0 (0.436 compared to  $0.23 \pm 0.09$  in normal controls) that was slightly higher than normal, but not consistent with a peroxisomal biogenesis disorder (PBD samples:  $3.93 \pm 1.5 \mu\text{g/ml}$ ) (Data in Brief Table 1). However, the total lipid fatty acid profile was abnormal, and a repeat sample at age 11 years was interpreted as normal. Indeed, the patient's C24/C22 ratio at 11 years was 0.901 ( $0.84 \pm 0.1$  in normal controls) and the C26/C22 was 0.02 ( $0.01 \pm 0.004$  in normal controls;  $0.5 \pm 0.16$  in PBD samples). An electromyogram and nerve conduction study (EMG/NCS) was performed and showed prolonged sensory and motor latency with slow conduction velocity consistent with a demyelinating neuropathy. Over the course of her teenage years, she underwent a progressive functional decline eventually becoming wheelchair bound at age 15 years (Data in Brief Fig. 1A). Specific DNA diagnostic tests for neurodegenerative disorders were uninformative including molecular testing for several forms of spinocerebellar ataxia (SCA1, SCA2, SCA7, SCA10), Friedreich ataxia (FRDA), spastic paraplegia (SPG3, SPG4, SPG8, SPG1), Pelizaeus–Merzbacher (PLP), pantothenate kinase deficiency (PANK2), Dentatorubral–pallidoluysian atrophy (DRPLA), cerebrotendinous xanthomatosis and several other leukodystrophies (GJA12, EIF2B5, EIF2B4, EIF2B2, EIF2B3, EID2B1) (Data in Brief Fig. 1B).

Her MRI studies (age 10 years and 19 years shown) showed evidence of diffuse T2 hyperintensity of her white matter (Fig. 1B versus a control MRI in Fig. 1A), which worsened with age (Fig. 1C). She also developed cerebellar volume loss, as evidenced by prominence of her folia (purple arrows, Fig. 1E versus a control MRI in Fig. 1D) which also worsened with age (Fig. 1F).

### 3.2. WES results

At age 21 years, whole exome sequencing was performed and it revealed a homozygous, three-nucleotide in frame deletion mutation in the *PEX16* gene causing an F332del (Fig. 1G). Both parents were heterozygous for this mutation. This mutation is at the extreme C-terminus of the *PEX16* protein within a cytoplasmic domain [6,23], and deletes a highly conserved phenylalanine (Additional variants in this case in Data in Brief Table 2).

This variant was not found within the ExAC browser data, [Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: <http://exac.broadinstitute.org>) accessed July 2015].

### 3.3. Peroxisomal biochemical analysis

At age 22 years plasma and fibroblast analysis of peroxisomal function was undertaken. The level of C26:0 was normal ( $0.31 \mu\text{g/ml}$ , Normal controls  $0.23 \pm 0.09$ ), with a subtle increase in the ratio of C24/C22 and C26/C22 (Z-scores 3.75 and 3.5 respectively, compared to 12.3 and 122.5 for PBD samples) (Fig. 1H–I).

She had no evidence of defects in plasmalogen synthesis (Fig. 1J), phytanic acid oxidation (Fig. 1K), nor pristanic acid oxidation (Fig. 1L). Moreover, the plasma bile acids, catalase solubility and lyso phosphatidylcholines were also normal (Data in Brief Fig. 2A–C). There was a higher C26/C22 ratio in fibroblasts (Z-score 5.0) suggesting a VLCFA defect, although this was significantly milder than what has been observed in patients with PBD (Z-score 38.0). (Fig. 1M).

## 4. Discussion

Our patient represented a diagnostic unknown prior to WES. She underwent a progressive neurodegeneration, and screening of plasma VLCFA's did not suggest PBD initially. Her diagnostic odyssey reflected a patient with a previously undescribed ataxic leukodystrophy. It was only after WES that a PBD could be identified. Our patient's clinical phenotype was retrospectively similar to cases of an unusual variant disorder with progressive paraparesis and ataxia due to *PEX16*

mutations recently reported including one patient with a mutation in an adjacent amino acid to that of our patient (Fig. 1G) [3]. However, in contrast to our patient, these individuals were identified based on plasma VLCFA abnormalities. Indeed, our patient's plasma C26/C22 ratio in three measurements was 0.022, 0.02 and 0.024 at 10, 11 and 22 years respectively, while the 6 other cases of atypical disorders due to *PEX16* mutations had slightly higher ratios (range 0.03–0.25) [3]. It remains to be determined how common these atypical peroxisomal are, but clearly in this case next-generation sequencing was necessary for diagnosis because of mild plasma biochemical analytes.

## 5. Conclusion

This case points to the value of next-generation sequencing in ataxia phenotypes, when biochemical measurements of peroxisomal pathways are mild. Mutations in *PEX16* and other *PEX* loci may underlie similar undiagnosed ataxia phenotypes and further studies are needed into these atypical cases.

## Acknowledgements

The authors thank Ann Snowden at KKI for cell culture technical support and Hugo Bellen for helpful advice in data analysis. Cell culture work funded by the Intellectual and Developmental Disability Research Center 1 U54 HD079123-01A1 at KKI PI: Wayne Silverman funded by: NICHD. M.W. was supported by NIH K08NS076547 funded by NINDS, and Funding from the Simmons Family Foundation Collaborative Research Fund and the Clayton Murphy Peroxisomal Disorders Research Fund at Baylor College of Medicine.

## References

- [1] M.N. Bainbridge, W. Wiszniewski, D.R. Murdock, J. Friedman, C. Gonzaga-Jauregui, et al., Whole-genome sequencing for optimized patient management, *Sci. Transl. Med.* 3 (2011) 87re83.
- [2] P. Danecek, A. Auton, G. Abecasis, C.A. Albers, E. Banks, et al., The variant call format and VCFtools, *Bioinformatics* 27 (2011) 2156–2158.
- [3] M.S. Ebberink, B. Csanyi, W.K. Chong, S. Denis, P. Sharp, et al., Identification of an unusual variant peroxisome biogenesis disorder caused by mutations in the *PEX16* gene, *J. Med. Genet.* 47 (2010) 608–615.
- [4] J. Gartner, N. Preuss, U. Brosius, M. Biermanns, Mutations in *PEX1* in peroxisome biogenesis disorders: G843D and a mild clinical phenotype, *J. Inher. Metab. Dis.* 22 (1999) 311–313.
- [5] C. Gonzaga-Jauregui, J.R. Lupski, R.A. Gibbs, Human genome sequencing in health and disease, *Annu. Rev. Med.* 63 (2012) 35–61.
- [6] M. Honsho, T. Hiroshige, Y. Fujiki, The membrane biogenesis peroxin Pex16p. Topogenesis and functional roles in peroxisomal membrane assembly, *J. Biol. Chem.* 277 (2002) 44513–44524.
- [7] W.C. Hubbard, A.B. Moser, A.C. Liu, R.O. Jones, S.J. Steinberg, et al., Newborn screening for X-linked adrenoleukodystrophy (X-ALD): validation of a combined liquid chromatography–tandem mass spectrometric (LC–MS/MS) method, *Mol. Genet. Metab.* 97 (2009) 212–220.
- [8] A. Imamura, S. Tamura, N. Shimozawa, Y. Suzuki, Z. Zhang, et al., Temperature-sensitive mutation in *PEX1* moderates the phenotypes of peroxisome deficiency disorders, *Hum. Mol. Genet.* 7 (1998) 2089–2094.
- [9] D.W. Johnson, H.J. ten Brink, R.C. Schuit, C. Jakobs, Rapid and quantitative analysis of unconjugated C(27) bile acids in plasma and blood samples by tandem mass spectrometry, *J. Lipid Res.* 42 (2001) 9–16.
- [10] R.M. Kok, L. Kaster, A.P. de Jong, B. Poll-The, J.M. Saudubray, et al., Stable isotope dilution analysis of piperolic acid in cerebrospinal fluid, plasma, urine and amniotic fluid using electron capture negative ion mass fragmentography, *Clin. Chim. Acta* 168 (1987) 143–152.
- [11] S.A. Lagerstedt, D.R. Hinrichs, S.M. Batt, M.J. Magera, P. Rinaldo, et al., Quantitative determination of plasma c8–c26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders, *Mol. Genet. Metab.* 73 (2001) 38–45.
- [12] C. Lamperti, M. Fang, F. Invernizzi, X. Liu, H. Wang, et al., A novel homozygous mutation in *SUCLA2* gene identified by exome sequencing, *Mol. Genet. Metab.* 107 (2012) 403–408.
- [13] J.R. Lupski, C. Gonzaga-Jauregui, Y. Yang, M.N. Bainbridge, S. Jhangiani, et al., Exome sequencing resolves apparent incidental findings and reveals further complexity of SH3TC2 variant alleles causing Charcot–Marie–Tooth neuropathy, *Genome Med.* 5 (2013) 57.
- [14] M.C. McGuinness, A.B. Moser, B.T. Poll-The, P.A. Watkins, Complementation analysis of patients with intact peroxisomes and impaired peroxisomal beta-oxidation, *Biochem. Med. Metab. Biol.* 49 (1993) 228–242.

- [15] A. Mignarri, C. Vinciguerra, A. Giorgio, S. Ferdinandusse, H. Waterham, et al., Zellweger spectrum disorder with mild phenotype caused by PEX2 gene mutations, *JIMD Rep.* 6 (2012) 43–46.
- [16] A.B. Moser, N. Kreiter, L. Bezman, S. Lu, G.V. Raymond, et al., Plasma very long chain fatty acids in 3,000 peroxisome disease patients and 29,000 controls, *Ann. Neurol.* 45 (1999) 100–110.
- [17] S.B. Ng, D.A. Nickerson, M.J. Bamshad, J. Shendure, Massively parallel sequencing and rare disease, *Hum. Mol. Genet.* 19 (2010) R119–R124.
- [18] G. Novarino, A.G. Fenstermaker, M.S. Zaki, M. Hofree, J.L. Silhavy, et al., Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders, *Science* 343 (2014) 506–511.
- [19] A. Poulos, Diagnosis of Refsum's disease using [1–14C]phytanic acid as substrate, *Clin. Genet.* 20 (1981) 247–253.
- [20] L. Regal, M.S. Ebberink, N. Goemans, R.J. Wanders, L. De Meirleir, et al., Mutations in PEX10 are a cause of autosomal recessive ataxia, *Ann. Neurol.* 68 (2010) 259–263.
- [21] A. Roscher, B. Molzer, H. Bernheimer, S. Stockler, I. Mutz, et al., The cerebrohepato renal (Zellweger) syndrome: an improved method for the biochemical diagnosis and its potential value for prenatal detection, *Pediatr. Res.* 19 (1985) 930–933.
- [22] C. Sevin, S. Ferdinandusse, H.R. Waterham, R.J. Wanders, P. Aubourg, Autosomal recessive cerebellar ataxia caused by mutations in the PEX2 gene, *Orphanet J. Rare Dis.* 6 (2011) 8.
- [23] S.T. South, S.J. Gould, Peroxisome synthesis in the absence of preexisting peroxisomes, *J. Cell Biol.* 144 (1999) 255–266.
- [24] A. Untergasser, I. Cutcutache, T. Koressaar, J. Ye, B.C. Faircloth, et al., Primer3—new capabilities and interfaces, *Nucleic Acids Res.* 40 (2012), e115.
- [25] R.J. Wanders, Metabolic and molecular basis of peroxisomal disorders: a review, *Am. J. Med. Genet. A* 126A (2004) 355–375.
- [26] R.J. Wanders, S. Denis, J.P. Ruiters, R.B. Schutgens, C.W. van Roermund, et al., Measurement of peroxisomal fatty acid beta-oxidation in cultured human skin fibroblasts, *J. Inher. Metab. Dis.* 18 (Suppl. 1) (1995) 113–124.
- [27] R.J. Wanders, H.R. Waterham, Peroxisomal disorders I: biochemistry and genetics of peroxisome biogenesis disorders, *Clin. Genet.* 67 (2005) 107–133.
- [28] P.A. Watkins, M.C. McGuinness, G.V. Raymond, B.A. Hicks, J.M. Sisk, et al., Distinction between peroxisomal bifunctional enzyme and acyl-CoA oxidase deficiencies, *Ann. Neurol.* 38 (1995) 472–477.
- [29] Y. Yang, D.M. Muzny, J.G. Reid, M.N. Bainbridge, A. Willis, et al., Clinical whole-exome sequencing for the diagnosis of Mendelian disorders, *N. Engl. J. Med.* 369 (2013) 1502–1511.