Parkinsonism and Related Disorders 65 (2019) 256–260

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Short communication

Chinese patients with adrenoleukodystrophy and Zellweger spectrum disorder presenting with hereditary spastic paraplegia

Yi-Jun Chen[a,1], Meng-Wen Wang[a,1], En-Lin Dong[a], Xiao-Hong Lin[a], Ning Wang[a,b], Zai-Qiang Zhang[c], Xiang Lin[a,b,**], Wan-Jin Chen[a,b,*]

[a] Department of Neurology and Institute of Neurology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, 350005, China
[b] Fujian Key Laboratory of Molecular Neurology, Fujian Medical University, Fuzhou, 350005, China
[c] Department of Neurology, Beijing Tiantan Hospital, Captical Medical University, Beijing, 100070, China

ARTICLE INFO

Keywords:
Peroxisomal disease
X-linked adrenoleukodystrophy
Zellweger spectrum disorder
ABCD1
PEX16
very long chain fatty acids
Magnetic resonance imaging

ABSTRACT

Introduction: X-linked adrenoleukodystrophy (ALD) and Zellweger spectrum disorder (ZSD) are peroxisomal diseases characterized by accumulation of very long chain fatty acids (VLCFA) in plasma and tissues. Considering the wide variability of manifestation, patients of ALD and atypical ZSD are easily misdiagnosed as hereditary spastic paraplegia (HSP) on their clinical grounds. Here, we aimed to determine the frequency of peroxisome diseases and compare their phenotypic spectra with HSP.

Methods: We first applied targeted sequencing in 120 pedigrees with spastic paraplegia, and subsequently confirmed 74 HSP families. We then performed whole exome sequencing for the probands of the 46 remaining pedigrees lacking known HSP-causal genes. Detailed clinical, radiological features, and VLCFA analyses are presented.

Results: Seven ALD pedigrees with *ABCD1* mutations and one ZSD family harboring bi-allelic mutations of *PEX16* were identified. Clinically, in addition to spastic paraplegia, four ALD probands presented adrenocortical insufficiency, and the ZSD proband and her affected sister both developed thyroid problems. VLCFA analysis showed that ratios of C24/C22 and C26/C22 were specifically increased in ALD probands. Moreover, three ALD probands and the ZSD proband had abnormalities in brain or spinal imaging.

Conclusions: Our study reports the first ZSD case in China that manifested spastic paraplegia, and emphasized the finding that peroxisomal diseases comprise a significant proportion (8/120) of spastic paraplegia entities. These findings extend our current understanding of the ALD and ZSD diseases.

1. Introduction

X-linked adrenoleukodystrophy (ALD) and Zellweger spectrum disorder are classified as peroxisomal diseases, which are characterized by the accumulation of very long chain fatty acids (VLCFA) in plasma and tissues [1]. ALD is caused by mutations of the ATP-binding cassette subfamily D member 1 (*ABCD1*) gene located on the chromosome Xq28 [2]. ALD can present at different ages, and clinical features provide distinctions in several sub-types: (i) Addison's only; (ii) cerebral ALD, a rapidly progressive form most frequently seen childhood; (iii) adrenomyeloneuropathy (AMN), an adult-onset form characterized by progressive spastic paraparesis, sensory dysfunction, and urinary symptoms; and (iv) symptomatic female heterozygotes [2]. AMN, the most common form, is clinically indistinguishable from hereditary spastic paraplegia (HSP) [3], especially when the inheritance pattern does not fit an X-linked mode [4]. In systematic screening studies, ALD has been considered the most frequent metabolic HSP [3,5].

Zellweger spectrum disorder (ZSD) represents the major subgroup within the peroxisomal (PEX) biogenesis disorders caused by mutations in any of 14 known *PEX* genes [6]. The severely affected individuals typically present in the neonatal period with brain dysfunction, distinct facial features, ocular abnormalities, sensorineural deafness, hepatic dysfunction, and (often) failure to thrive. Moreover, atypical phenotypes (e.g., progressive spastic paraplegia, ataxia, and dystonia) have also been characterized in the majority of the reported patients [7]. Considering the clinical overlap between ZSD and autosomal recessive

* Corresponding author. Department of Neurology and Institute of Neurology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, 350005, China.
** Corresponding Author. Department of Neurology and Institute of Neurology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, 350005, China.
E-mail addresses: linxiang1988@fjmu.edu.cn (X. Lin), wanjinchen75@fjmu.edu.cn (W.-J. Chen).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.parkreldis.2019.06.008
Received 11 March 2019; Received in revised form 7 June 2019; Accepted 8 June 2019

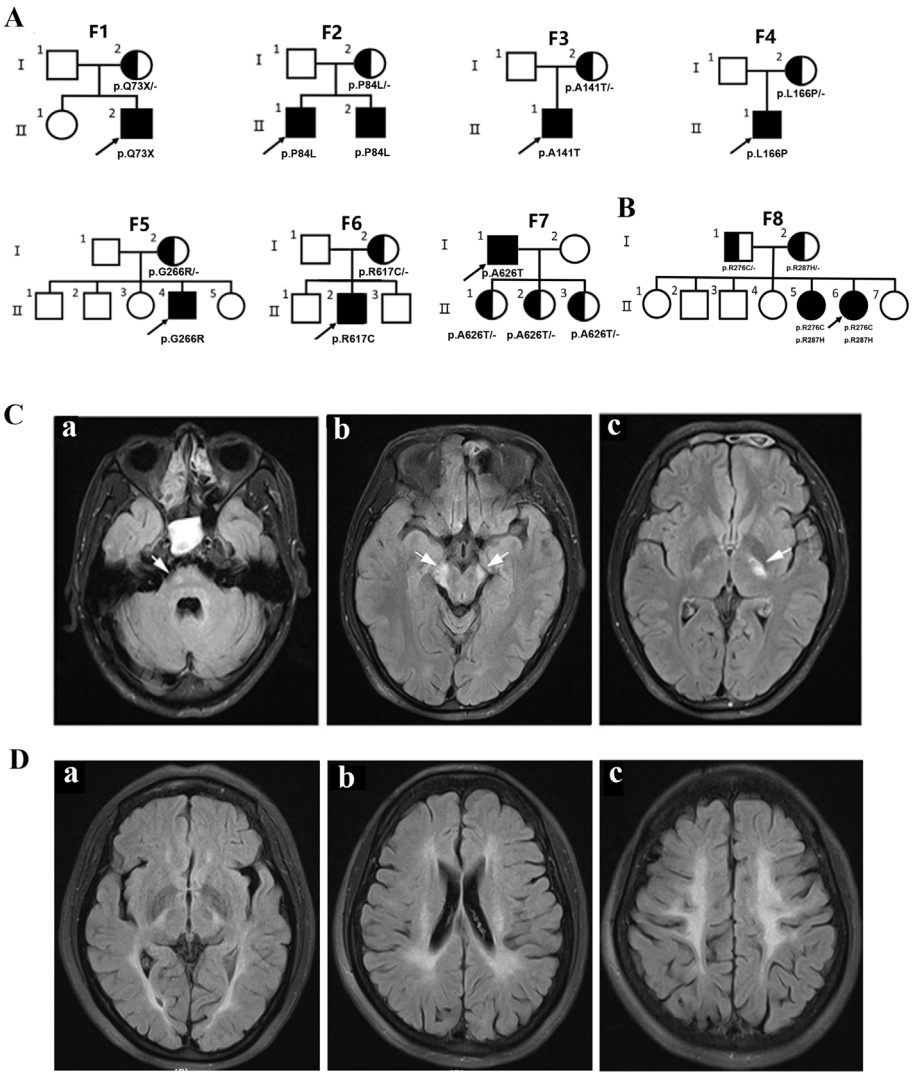

Fig. 1. Sequencing data and brain MRI for the ALD and ZSD families. (**A**) The mutations of *ABCD1* in the available members of seven ALD families (F1–F7). (**B**) The bi-allelic mutations of *PEX16* in the available members of ZSD family. Squares indicate males; circles indicate females; the black symbols indicate affected individuals; the half black symbols indicate carriers; arrows indicate the probands; dash symbols indicate reference allele. (**C**) Proband (II-2) in F1: arrows indicated increased signal intensities on axial T2-weighted FLAIR MR images in the right ventral pons (a), bilateral cerebral peduncles (b), and posterior limb of the left internal capsule (c). (**D**) Proband (II-6) in F8: bilaterally symmetric increased signal intensities on axial T2-weighted FLAIR MR images in the posterior limb of internal capsule (a), periventricular area (b), and centrum semiovale (c).

HSP, differentiation between them can be difficult based solely on their clinical grounds. In particular, ZSD patients with atypical onset of spastic paraplegia are thus easily misdiagnosed as cases of complicated HSP.

To further determine the frequency of peroxisome diseases and to compare their phenotypic spectra with HSP, we applied whole exome sequencing (WES) to probands of 46 HSP families that still lacked known HSP-causative genes when screened by targeted sequencing. A combination of genetic screening and biochemical testing confirmed that 7 male probands were AMN and one female proband was ZSD. Our study thus serves as a proof-of-principle that clinical evaluation, genetic basis, and biochemical analysis applied in concert are all necessary for their separate contributions in distinguishing peroxisomal diseases from HSP.

2. Patients and methods

2.1. Subject recruitment

In total, 120 pedigrees fulfilling the clinical diagnostic criteria for HSP [8] were recruited from the Department of Neurology, First Affiliated Hospital of Fujian Medical University. The neurological examination and clinical evaluations were performed by at least two senior neurologists. Written informed consent was obtained from all the participants. This study was approved by the ethics committees of the First Affiliated Hospital of Fujian Medical University.

2.2. Sample collection and targeted sequencing

Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). A panel of targeted sequencing was designed to cover 149 genes known to be correlated with HSP.

2.3. WES sequencing and data analysis

WES was performed for the probands of the 46 remaining pedigrees that still lacked known HSP-causative genes after screening by the aforementioned targeted sequencing. Whole exomes were captured using a SeqCap EZ Exome Kit V3 (Roche Technologies) and sequenced using the Illumina HiSeq 3000 platform. Sequencing reads were obtained in the Fastq format. The reads were mapped to human genome GRCh38 using Burrows Wheeler Aligner. Variants were called using the Genome Analysis Toolkit and annotated with ANNOVAR. Variants that met the following criteria were excluded first: i) the variant did not affect a change in amino acid sequence; and ii) the allele frequency was > 1% in the 1000 Genomes Project, ESP database, or gnomAD.

All the variants were further filtered by Sorting intolerant from tolerant (SIFT), Polymorphism phenotyping-2 (PolyPhen-2), and Mutation Taster database.

Table 1
Summary of clinical features of the eight probands of ALD and ZSD families.

Probands No.	II-2 (F1)	II-1 (F2)	II-1 (F3)	II-1 (F4)	II-4 (F5)	II-2 (F6)	I-1 (F7)	II-6 (F8)
Gender	M	M	M	M	M	M	M	F
Age at onset	28	30	40	22	23	25	50	32
Initial symptoms	Weakness of legs; spastic gait; tremor of the right leg	Spastic gait	Weakness of legs; spastic gait	Spastic gait	Weakness and stiffness of legs; spastic gait	Stiffness of legs	Weakness of legs; spastic gait	Weakness of legs; spastic gait; tremor of hands
Dysarthria	No	No	Yes	No	No	No	No	Yes
Sphincter dysfunction	Yes	Yes	No	Yes	Yes	No	No	No
Hypoadrenocorticism	Yes	Yes	No	Yes	Yes	No	No	No
Other symptoms	No	No	No	No	No	No	No	Thyroiditis
Muscle strength*-LL	5$^-$	5	5$^-$	4$^-$	2	5	4$^-$	4
Hypertonia-LL	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Reflex-UL	+ + +	+ +	+ +	+ + +	+ +	+ + +	+ + +	+ + +
Reflex-LL	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + + +	+ + + +
Babinski's sign (UL)	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive
Babinski's sign (LL)	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Positive
Sensory	Normal	Normal	Impaired vibration sense	Hypoesthesia	Impaired deep sense	Normal	Normal	Normal
Cerebellar sign	Truncal ataxia	Limb ataxia	Slurred speech	No	No	No	Truncal ataxia	Limb ataxia; Slurred speech
Brain MRI	Abnormal	Normal	Normal	NA	Normal	Normal	NA	Abnormal
Spinal cord MRI	Normal	Normal	Normal	Atrophy	Normal	Atrophy	Normal	NA

M, male; F, female; LL: lower limb; UL: upper limb; +: decreased reflex; + +: normal deep reflex; + + +: brisk reflex; + + + +: hyperreflexia; NA: not available. *Muscle strength was graded on a modified Medical Research Council Scale (D.M. Rosanne, D.P. Fredrick, Preparing for The Occupational Therapy Assistant National Board Exam: 45 Days and Counting, in: H. Tia (Eds.), Jones & Bartlett Learning, LLC, 2017, pp. 58.).

2.4. Sanger sequencing

Sanger sequencing was carried out to validate the potential variants identified by WES on an ABI 3500xL DxGenetic Analyzer (Applied Biosystems, Foster City, USA). All available familial members with or without HSP were screened for co-segregation analysis. Specific primers used for amplification were listed in Supplementary Table 1.

2.5. VLCFA in plasma analysis

EDTA whole blood was extracted from fasting participants and plasma VLCFA levels were measured using Gas Chromatography Mass Spectrometry.

3. Results

3.1. Identification of variants by WES and Sanger sequencing

Targeted sequencing was performed in 120 pedigrees, leading to the identification of 74 pedigrees that harbored known, causative genes of HSP (Supplemental Table 2). We then applied WES to the index cases of the 46 remaining pedigrees that still lacked a genetic diagnosis. After verification by bioinformatic analysis and Sanger sequencing, we ultimately identified two peroxisomal diseases, ALD and ZSD, in eight families, and also revealed Alzheimer's disease-3 and X-linked spinocerebellar ataxia in two additional families (data available). Among the previously mentioned eight families with peroxisomal disease, seven ALD families carried seven different variants of the *ABCD1* (NM_000033.4) (Fig. 1A), and a ZSD family had bi-allelic novel variants of the *PEX16* (NM_057174.2) (Fig. 1B). Notably, there was an unusual phenomenon in the Sanger sequencing results of the proband's mother (I-2) in family 5 (F5). Molecular analysis of white blood cell DNA did not reveal her status as an “obligate” carrier; however, after careful review of the electropherograms (Supplemental Fig. 1), we identified signal peaks for the mutant allele in both forward and reverse sequencing directions. As gonadal or gonosomal mosaicism has been reported previously in ALD [9], we thus speculated that the mother possessed a comparatively rare mosaicism.

Among the seven *ABCD*1 variants, five of them (c.251C > T, c.421G > A, c.796G > A, c.1849C > T, and c.1876G > A) have been documented as pathogenic in ALD patients and are found in the Human Gene Mutation Database. The other two variants (c.217C > T and c.497T > C) were novel and absent from the ExAc and 1000G databases. Variant c.497T > C was predicted to be damaging by SIFT, PolyPhen-2 and Mutation Taster. The two *PEX16* variants (c.826C > T and c.860G > A), also absent in ExAc and 1000G, were similarly predicted to be deleterious. According to the standards of the American College of Medical Genetics and Genomics [10], variant c.217C > T was classified as pathogenic, while variant c.497T > C, as well as was both *PEX16* variants, were all classified as likely pathogenic mutations.

3.2. VLCFA analysis in ALD and ZSD families

After genetic screening by WES, the plasma VLCFA levels of the patients and the available family members were also measured, resulting in strongly supported diagnoses. The mean ± SD of all items from patients and heterozygotes are summarized in Supplemental Table 3. Among all of the items measured, C26:0 was deemed most effective for identification of AMN patients or heterozygous carriers, since it was elevated in all patients as well as in a majority of the heterozygous carriers. Additionally, the ratios of both C24/C22 and C26/C22 in patients was much higher than in carriers. These observations revealed that the combined evaluation of C24/C22 and C26/C22 ratios was effective for diagnosis of AMN patients.

3.3. Clinical characterization of ALD and ZSD patients

The detailed clinical features of the seven probands from each of the ALD families are summarized in Table 1. These patients were all male, and the age of onset ranged from 20 to 50 years old. All of them complained of stiffness in their lower limbs associated with spastic gait, and the proband (II-2) of family 1 (F1) also presented with tremors of the right leg. Proband (II-1), a 40-year-old male in family 2 (F2), had a positive family history and manifested with spastic paraplegia and sphincter dysfunction for 10 years. His 29-year-old brother (II-2) had the same but much milder neurological symptoms. Additionally, four

probands (families 1, 2, 4, and 5) had symptoms of adrenal insufficiency such as trichomadesis and skin pigmentation. Four probands (families 1, 2, 3, and 7) had obvious cerebellar signs (e.g., truncal or limb ataxia, slurred speech). Three probands (families 1, 4, and 6) showed abnormalities in brain or spinal imaging. Specifically, the brain MRI of proband (II-2) in F1 revealed increased signal intensities on axial T2-weighted fluid attenuated inversion recovery (FLAIR) in the right ventral pons, bilateral cerebral peduncles, and posterior limb of the left internal capsule (Fig. 1C). Since ALD carriers can develop an adrenomyeloneuropathy-like phenotype with increasing age [4], we also assessed the neurological symptoms and signs of female carriers of *ABCD1* mutations in our cohort. However, none of them presented with abnormal neurological symptoms or signs.

Proband (II-6), a 51-year-old female, in family 8 (F8) carried compound heterozygous mutations of the *PEX16*, and manifested gradually developed involuntary tremor and weakness of the upper limbs, accompanied by a severe spastic gait (Table 1). She had a six-year history of Hashimoto's thyroiditis. Several neurological signs were observed, including dysarthria, increased muscle tone and hyperreflexia of lower limbs, and bilateral positive extensor plantar responses. Her brain MRI examination displayed bilaterally symmetric increased signal intensities on axial T2-weighted FLAIR in the posterior limb of internal capsule, periventricular area, and centrum semiovale (Fig. 1D). Her 55-year-old sister (II-5) presented the same neurological symptoms for a 16-year history, and which were accompanied with thyroiditis that progressed to thyroid cancer. Unfortunately, she did not undergo brain MRI scanning.

4. Discussion

Progressive spasticity of lower limbs is a diagnostic challenge. Despite the fact that ALD is an X-linked recessive disease, it may present with spasticity in both sexes (e.g., carrier females) [3,4]. Although there was no obvious symptomatic status among female carriers in our cohort, long-term monitoring of their physiological conditions also warrants consideration.

The presence of the increased ratios of C24:0/C22:0 and C26:0/C22:0 in plasma is highly reliable for the diagnosis of ALD patients [2]. However, false negative results may happen in 15–20% of obligate carrier females [11]. Therefore, mutation screening of the *ABCD1* is considered to be the best way to uncover the female carriers' status. Notably, the presence of autosomal paralogous copies of exons 7 to 10 of *ABCD1* (on chromosomes 2p11,10p11,16p11, and 22q11) have led to complications in the molecular analysis [11]. We applied M13 tailed primers to specifically amplify *ABCD1* in order to validate the potential pathogenic mutations. Additionally, the rare occurrence of gonadal or gonosomal mosaicism should not be neglected in the targeted mutation analysis [9]. Since ALD is potentially treatable with steroids [2], the increased accuracy of genetic findings may also have implications for clinical management of this disease.

In pure AMN, the spinal cord is the principle site of pathology [2]. Quantitative MRI showed that the total cord area was significantly reduced at all examined levels (C1-T3) in AMN patients [5]. Intriguingly, 2 of 7 AMN patients only showed atrophy of the thoracic cord in our cohort. Previous studies revealed that ~20–60% of pure AMN patients developed additional cerebral demyelination and were referred to as AMN cerebral [2], which is distinguishable from cerebral ALD. The brain MR findings showed increased signal intensities in the projections of the pyramidal tract fibers in the pons, cerebral peduncles, and internal capsules on axial FLAIR and T2 sequences, which was consistent with the magnetic resonance performance of the proband (II-2) in family 1. Once the cerebral demyelinating lesions have entered the active neuroinflammation scored by the gadolinium enhancement intensity system, the prognosis is as poor as in childhood cerebral ALD [12].

Clinically, ZSD is highly heterogeneous. Patients with ZSD can present symptoms ranging from severe in their neonatal period to only minor features during their adolescence or adulthood [6,7]. In our study, we reported on two adult patients in family 8 who carried biallelic novel mutations in *PEX16*, who suffered from severe spastic gait, involuntary tremor, and dysarthria, and manifested white matter abnormalities consistent with ZSD. It is noteworthy that both of them developed thyroid problems, which implied the condition was not occasional but rather the byproduct of disease progression.

In conclusion, our study indicated that peroxisomal diseases represent a significant portion (8/120) of the spastic paraplegia entities, and that the combination of WES and plasma VLCFA analysis can be used for differential diagnoses, given the wide utility and availability of these methods. Likewise, as cerebral involvement is a poor prognostic indicator, brain MRI examination should be routinely performed to monitor the disease progression during follow-up.

Authors' roles

YJC: data acquisition, statistical analysis and interpretation, and manuscript preparation. MWW: data acquisition, analysis and interpretation. ELD: data acquisition and analysis. XHL: data acquisition and analysis. NW: data analysis and critical revision of the manuscript. ZQZ: data acquisition. XL: data acquisition, statistical analysis and interpretation, and critical revision of the manuscript. WJC: study design and conceptualization, data acquisition, analysis and interpretation, critical revision of the manuscript. All authors have approved the final article.

Funding

This work was supported by the grant 81771230 (W.-J.C.), 81801130 (X.L.), and U1505222 (N.W.) from the National Natural Science Foundation of China, grant 2017XQ1072 from Startup Fund for scientific research of Fujian Medical University (X.L.), grant 2019J05076 (X.L.) from the Natural Science Foundation of Fujian Province, Joint Funds for the Innovation of Science and Technology of Fujian Province (2017Y9094) (W.-J.C.), National Key Clinical Specialty Discipline Construction Program (N.W.), and Key Clinical Specialty Discipline Construction Program of Fujian (N.W.).

Financial disclosures/conflict of interest

All authors reported no disclosures.

Data statement

Any data not published within this article are available from the corresponding author on reasonable request.

Acknowledgments

The authors sincerely thank the participants for their help and willingness to participate in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.06.008.

References

[1] B.T. Poll-The, J. Gärtner, Clinical diagnosis, biochemical findings and MRI spectrum of peroxisomal disorders, Biochim. Biophys. Acta 1822 (9) (2012) 1421–1429.
[2] M. Engelen, S. Kemp, M. de Visser, B.M. van Geel, R.J. Wanders, P. Aubourg, B.T. Poll-The, X-linked adrenoleukodystrophy (X-ALD): clinical presentation and guidelines for diagnosis, follow-up and management, Orphanet J. Rare Dis. 7 (2012) 51.
[3] J. Müller vom Hagen, K.N. Karle, R. Schüle, I. Krägeloh-Mann, L. Schöls,

Leukodystrophies underlying cryptic spastic paraparesis: frequency and phenotype in 76 patients, Eur. J. Neurol. 21 (7) (2014) 983–988.
[4] M. Engelen, M. Barbier, I.M. Dijkstra, R. Schür, R.M. de Bie, C. Verhamme, M.G. Dijkgraaf, P.A. Aubourg, R.J. Wanders, B.M. van Geel, M. de Visser, B.T. Poll-The, S. Kemp, X-linked adrenoleukodystrophy in women: a cross-sectional cohort study, Brain 137 (Pt 3) (2014) 693–706.
[5] A. Castellano, N. Papinutto, M. Cadioli, G. Brugnara, A. Iadanza, G. Scigliuolo, D. Pareyson, G. Uziel, W. Köhler, P. Aubourg, A. Falini, R.G. Henry, L.S. Politi, E. Salsano, Quantitative MRI of the spinal cord and brain in adrenomyeloneuropathy: in vivo assessment of structural changes, Brain 139 (Pt 6) (2016) 1735–1746.
[6] F.C. Klouwer, K. Berendse, S. Ferdinandusse, R.J. Wanders, M. Engelen, B.T. Poll-The, Zellweger syndromes: clinical overview and management approach, Orphanet J. Rare Dis. 10 (2015) 151.
[7] N.A. Zaabi, A. Kendi, F. Al-Jasmi, S. Takashima, N. Shimozawa, O.Y. Al-Dirbashi, Atypical PEX16 peroxisome biogenesis disorder with mild biochemical disruptions and long survival, Brain Dev. 41 (1) (2019) 57–65.
[8] E.L. Dong, C. Wang, S. Wu, Y.Q. Lu, X.H. Lin, H.Z. Su, M. Zhao, J. He, L.X. Ma, N. Wang, W.J. Chen, X. Lin, Clinical spectrum and genetic landscape for hereditary spastic paraplegias in China, Mol. Neurodegener. 13 (1) (2018) 36.
[9] Y. Wang, R. Busin, C. Reeves, L. Bezman, G. Raymond, C.J. Toomer, P.A. Watkins, A. Snowden, A. Moser, S. Naidu, G. Bibat, S. Hewson, K. Tam, J.T. Clarke, L. Charnas, G. Stetten, B. Karczeski, G. Cutting, S. Steinberg, X-linked adrenoleukodystrophy: ABCD1 de novo mutations and mosaicism, Mol. Genet. Metab. 104 (1–2) (2011) 160–166.
[10] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, ACMG laboratory quality assurance committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and Genomics and the association for molecular pathology, Genet. Med. 17 (5) (2015) 405–424.
[11] C.D. Boehm, G.R. Cutting, M.B. Lachtermacher, H.W. Moser, S.S. Chong, Accurate DNA-based diagnostic and carrier testing for X-linked adrenoleukodystrophy, Mol. Genet. Metab. 66 (2) (1996) 128–136.
[12] W.P. Miller, L.F. Mantovani, J. Muzic, J.B. Rykken, R.S. Gawande, T.C. Lund, R.M. Shanley, G.V. Raymond, P.J. Orchard, D.R. Nascene, Intensity of MRI gadolinium enhancement in cerebral adrenoleukodystrophy: a biomarker for inflammation and predictor of outcome following transplantation in higher risk patients, AJNR (Am. J. Neuroradiol.) 37 (2) (2016) 367–372.