Molecular Genetics and Metabolism Reports 25 (2020) 100694

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

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

Zellweger spectrum disorder: A cross-sectional study of symptom prevalence using input from family caregivers

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ARTICLE INFO

Keywords:
Peroxisome biogenesis disorder
Rare disease
Surveys and questionnaires
Cross-sectional study
Symptom prevalence
Caregiver report
Zellweger spectrum disorders

ABSTRACT

Zellweger spectrum disorders (ZSD) are rare, debilitating genetic diseases of peroxisome biogenesis that affect multiple organ systems and present with broad clinical heterogeneity. Although many case studies have characterized the multitude of signs and symptoms associated with ZSD, there are few reports on the prevalence of symptoms to help inform the development of meaningful endpoints for future clinical trials in ZSD. In the present study, we used an online survey tool completed by family caregivers to study the occurrence, frequency and severity of symptoms in individuals diagnosed with ZSD. Responses from caregivers representing 54 living and 25 deceased individuals with ZSD were collected over an 8-month period. Both perception of disease severity and prevalence of various symptoms were greater in responses from family caregivers of deceased individuals compared to those of living individuals with ZSD. Compared with previous reports for ZSD, the combined prevalence of seizures (53%) and adrenal insufficiency (45%) were nearly twice as high. Overall, this community-engaged approach to rare disease data collection is the largest study reporting on the prevalence of symptoms in ZSD, and our findings suggest that previous reports may be underreporting the true prevalence of several symptoms in ZSD. Studies such as this used in conjunction with clinician- led reports may be useful for informing the design of future clinical trials addressing ZSD.

1. Introduction[1]

Peroxisomes are membrane-bound organelles in almost all eukaryotic cells. Mature peroxisomes contain multiple enzymes required for diverse biochemical processes, including a variety of lipid metabolic pathways [1]. Inherited peroxisomal disorders in humans are often attributed to single enzyme defects within the peroxisome or disorders of overall peroxisome biogenesis, which result in defective biosynthesis, assembly, and general functionality of peroxisomes. Peroxisome biogenesis disorders (PBDs) are primarily caused by mutations in any of 14 different *PEX* genes, which code for peroxins, proteins involved in peroxisome assembly and importation of peroxisomal matrix proteins [2]. PBDs are categorized into two groups of diseases: rhizomelic chondrodysplasia punctata [3] and Zellweger spectrum disorders.

Zellweger spectrum disorders (ZSD) are autosomal recessive disorders with a cumulative incidence of ~1:50,000 births [4,5]. ZSD

Abbreviations: DMCC, Data Management and Coordinating Center; DXA, dual-energy x-ray absorptiometry; EEG, Electroencephalogram; GFPD, Global Foundation for Peroxisomal Disorders; MRI, Magnetic Resonance Imaging; PBDs, Peroxisome biogenesis disorders; RDCRN, Rare Diseases Clinical Research Network; STAIR, Sterol and Isoprenoid Research Consortium; ZSD, Zellweger Spectrum Disorder.

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https://doi.org/10.1016/j.ymgmr.2020.100694
Received 27 November 2020; Accepted 28 November 2020
Available online 10 December 2020

patients present with multi-organ symptoms and extensive clinical heterogeneity. Cardinal manifestations include low muscle tone, facial dysmorphisms, impaired growth, sensory and neurological dysfunction, renal and endocrine insufficiency, skeletal abnormalities, and developmental delays [6–19]. Many ZSD symptoms are present at birth or appear in early childhood; most ZSD symptoms are progressive in nature. The most severe forms of ZSD are fatal in early childhood, but patients with milder forms of ZSD can survive into adulthood.

The small sample sizes in the majority of clinical reports on ZSD, in conjunction with the extensive phenotypic heterogeneity, present challenges in drawing conclusions about the severity and frequency of symptoms, such as seizures [6,20].

Natural history studies with in-person study visits at clinical sites are foundational for understanding disease variation and progression, ultimately to guide clinical trial development. However, in the case of rare diseases, clinic-based studies are limited by the small number of patients willing to enroll, by poor compliance to regular study visit schedules, and in the case of debilitating conditions, by the logistical challenge of traveling to distant study sites, which may preclude the collection of comprehensive data for optimal disease management and therapeutic trial design.

The use of online, self-reported data in rare disease research can be a valuable resource to provide aggregate information on symptoms as well as treatment modalities, with relatively low barriers to participation compared to clinician-led studies [21,22]. For example, an online survey of 1,057 Duchenne muscular dystrophy patients across the United States confirmed that steroid use in patients was associated with a prolonged capacity to walk. Additionally, the same study found that steroid use was actually higher in patients than previously reported in clinical trials with smaller sample sizes [23]. Studies such as this highlight the potential value of patient- reported data in providing a rich characterization of the patient experience vis-à-vis clinical observation. Moreover, for ZSD patients who cannot self-report due to functional and communication limitations, the Food and Drug Administration as well as independent task forces have provided guidance on the creation of observer-reported outcome assessments [24,25]. Among rare pediatric diseases that have used observer-reported outcome instruments, most are dependent on the report of the primary family caregiver [26,27].

Recognizing the need for more information about the frequency and severity of ZSD symptoms to assist clinical management and help inform future clinical trials, we sought the input of family caregivers (parents, stepparents and legal guardians) of individuals diagnosed with ZSD using an online observer-reported survey instrument. We partnered with the Global Foundation for Peroxisomal Disorders (GFPD, http://www.thegfpd.org), a patient advocacy group focused on ZSD and related peroxisomal disorders, to conduct this study. The GFPD is one of the advocacy groups partnering with the Sterol and Isoprenoid Research (STAIR) Consortium, a consortium within the NIH/NCATS-funded Rare Diseases Clinical Research Network (RDCRN). RDCRN resources included consortium-specific contact registries managed by the RDCRN data management and coordinating center (DMCC) at the University of South Florida. The STAIR contact registry and DMCC were leveraged for this study to facilitate patient recruitment and support data collection with online surveys.

2. Materials and methods

2.1. ZSD Symptom Inventory

The novel ZSD Symptom Inventory was developed for caregivers of individuals with ZSD and related disorders, with the assistance and input from GFPD families and members of the GFPD scientific advisory board. The ZSD Symptom Inventory is an online survey with multiple-choice and open-ended questions, initially based on clinical intake questions used in a previously published study [28]. Pilot testing of the survey was conducted at a GFPD family conference, where caregivers were asked to complete a draft version of the survey on their electronic and mobile devices and subsequently provide feedback. Feedback was required on both the questions and the response to questions. A 77-item survey was finalized, with questions about symptoms, medical assessment results and treatment, organized by the following domains: diagnosis, muscle tone and mobility, communication and sensory systems, bone/dental/endocrine system, neurology, psychosocial symptoms and gastrointestinal system. Questions regarding symptoms were primarily presented as “Does/did your child have [given symptom]?” followed by three possible item responses: yes, no, or I don’t know. (See Supplementary Material Table 1 for full survey instrument).

2.2. Recruitment

Approval for the study was granted by the University of South Florida Institutional Review Board (USF IRB Pro00033243). To be eligible, participants needed to be enrolled in the STAIR contact registry as the family caregiver (parent, step-parent, or legal guardian) to at least one individual (living or deceased) diagnosed with either ZSD, D-bifunctional protein deficiency, or acyl CoA oxidase (ACOX) deficiency. Participants with multiple children affected with ZSD or a related peroxisomal disorder registered each of their children individually in the registry. The DMCC sent an initial call for participation via email on February 1, 2018, to all registry members eligible for the study. The invitation contained a hyperlink to the informed consent form for the study and questionnaire. The GFPD further supported recruitment through e-mail announcements to its members, social media posts, and mailings. These notices encouraged family caregivers to both sign up in the STAIR Contact registry and participate in the study.

Participants were asked to complete the online survey through the STAIR Contact Registry after consent. Access to the online questionnaires/instruments was given at the time of consent. A link to the questionnaire unique to each participant was generated (with multiple links generated for participants with multiple children diagnosed with ZSD or a related peroxisome disorder), to account for who had initiated and completed the questionnaires. Investigators were blinded to the identity of the participants.

2.3. Data collection

Study participants entered their survey responses directly into online forms, either all at once or in multiple sessions. Two reminder emails were sent to those who had not started the survey or only partially completed the survey one month following completion of the consent and again one week before the study closed. All survey responses, including partial responses, were directly imported in the DMCC database for analysis. The study closed on September 30, 2018, with 92 consented responses. All project data were stored in a database that complied with all applicable guidelines regarding patient confidentiality and data integrity.

2.4. Statistical analyses

Statistical analyses were performed using SAS v9.4 (Cary, NC). Comparisons focused on group differences between participants self-identifying as parents of living individuals diagnosed with ZSD and those self-identifying as parents of deceased individuals diagnosed with ZSD. Based on the number of responses for each survey item across groups, Chi-square tests or Fisher’s exact tests were performed to test for differences between the groups. Differences with a p value < 0.05 were considered statistically significant with no adjustment for multiple testing.

3. Results

3.1. Demographic information

Of the 145 STAIR contact registry participants who received invitations, 92 responses were collected for the survey. Eighty-six respondents identified as the family caregiver of an individual with ZSD, and 6 as the family caregiver of an individual with D-bifunctional protein deficiency. Eight participants were family caregivers for multiple individuals with ZSD (7 participants with 2 children, 1 participant with 3 children); these participants were invited to submit a survey response representing each child and subsequently each of those responses were analyzed as an individual response. Seven responses were duplicate entries for the same individual with ZSD (*e.g.* both mother and the father reported on the same child) and therefore only one response was included in the analysis (primarily the entry with the most comprehensive survey response). Additionally, as there were only 6 participants reporting on an individual with D-bifunctional protein deficiency, these responses were excluded from the final analysis.

Ultimately, the final analysis included 79 responses (70 participants total), with 54 responses from individuals identifying as the family caregiver of a living individual diagnosed with ZSD, and 25 responses from individuals identifying as family caregivers of a deceased individual with ZSD (Supplementary material Table 2). Ninety percent of responses reported that they were white (n = 71), 4% reported that they black (n = 4), 1% that they were Asian (n = 1), 1% responded as multiracial (n = 1), and 3% of responses did not report their ethnic background (n = 2). The majority of the responses identified as mothers (81%) of individuals diagnosed with ZSD.

The median age for living individuals with ZSD at the time of the study was 7.2 years (Interquartile range: 4.7–17.7 years) Table 1. The median age for deceased individuals at the time of death was 1.2 years (Interquartile range: 0.6–9.7 years). Although respondents were permitted to skip survey question items, 87% (47/54) of family caregivers of living individuals with ZSD answered all pertinent survey items regarding symptoms, and 80% (20/25) of family caregivers of deceased individuals with ZSD answered all pertinent survey items.

3.2. Disease severity

Participants were asked "How would you describe the severity of your child's disorder?" and given the following choices for response items, "Mild, mild to intermediate, intermediate, intermediate to severe, and severe." Responses were significantly different between family caregivers of living individuals with ZSD and family caregivers of deceased individuals, with more caregivers of deceased individuals reporting that their child's disorder was "severe" compared to caregivers of living individuals (60% vs 7%, $p < 0.001$, Table 1).

3.3. Neurological symptoms

Participants were asked if their child had ever had an electroencephalogram (EEG) or magnetic resonance imaging (MRI) and subsequently asked if these assessments showed abnormal results. Among family caregivers of living individuals with ZSD, 47% (n = 15, out of 32 who indicated their child had an EEG) reported that their child's EEG was abnormal, while 89% of caregivers of deceased individuals (n = 17, out of 19 who indicated their child had an EEG) reported that their child's EEG was abnormal (p = 0.007, Table 1).

Regarding MRIs, 57% of family caregivers for living individuals with ZSD (n = 25, out of 32 who indicated their child had an MRI) reported that their child's MRI was abnormal, and 89% of caregivers of deceased individuals with ZSD (n = 16, out of 18 who indicated their child had an MRI) reported that their child's MRI was abnormal (p = 0.044, Table 1).

When participants were asked about seizures in their children, 7% (n = 4) of family caregivers for living individuals with ZSD reported that

Table 1
Symptom Prevalence in ZSD as Reported by Family Caregivers.

Status of patient	Total n (%)	Living n (%)	Deceased n (%)	P-Value
Median age in years (Interquartile Range)		7.2 (4.7–17.7)	1.2 (0.6–9.7)	
Severity				
Mild	10 (12.7)	10 (18.5)	0 (0.0)	
Mild to intermediate	12 (15.2)	11 (20.4)	1 (4.0)	
Intermediate	25 (31.6)	23 (42.6)	2 (8.0)	
Intermediate to severe	13 (16.5)	6 (11.1)	7 (28.0)	
Severe	19 (24.0)	4 (7.4)	15 (60.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	**<0.001**
Neurological symptoms				
Abnormal EEG				
Yes	32 (62.7)	15 (46.9)	17 (89.4)	
No	16 (31.4)	15 (46.9)	1 (5.3)	
Don't know	3 (5.9)	2 (6.2)	1 (5.3)	
Total	51 (100.0)	32 (100.0)	19 (100.0)	**0.007**
Abnormal MRI				
Yes	41 (66.1)	25 (56.8)	16 (88.9)	
No	15 (24.2)	13 (29.6)	2 (11.1)	
Don't know	6 (9.7)	6 (13.6)	0 (0.0)	
Total	62 (100.0)	44 (100.0)	18 (100.0)	**0.044**
Seizures				
Yes, 1 time	4 (5.0)	4 (7.4)	0 (0.0)	
Yes, multiple times	38 (48.1)	20 (37.0)	18 (72.0)	
No	30 (38.0)	25 (46.3)	5 (20.0)	
I don't know	7 (8.9)	5 (9.3)	2 (8.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	**0.025**
Balance problems (Ataxia)				
Yes	42 (53.2)	34 (63.0)	8 (32.0)	
No	18 (22.8)	10 (18.50)	8 (32.0)	
Don't know	19 (24.0)	10 (18.5)	9 (36.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	**0.037**
Sleep disturbances				
Yes	32 (41.0)	24 (44.4)	8 (33.3)	
No	40 (51.3)	27 (50.0)	13 (54.2)	
Don't know	6 (7.7)	3 (5.6)	3 (12.5)	
Total	78 (100.0)	54 (100.0)	24 (100.0)	0.450
Gastrointestinal symptoms				
Feeding difficulties				
Yes	60 (76.0)	35 (64.8)	25 (100.0)	
No	19 (24.0)	19 (35.2)	0 (0.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	**0.001**
Gastroesophageal reflux				
Yes	27 (34.2)	12 (22.2)	15 (60.0)	
No	46 (58.2)	38 (70.4)	8 (32.0)	
Don't know	6 (7.6)	4 (7.4)	2 (8.0)	
Total		54 (100.0)		**0.003**

(*continued on next page*)

Table 1 (*continued*)

Status of patient	Total n (%)	Living n (%)	Deceased n (%)	P-Value
	79 (100.0)		25 (100.0)	
Abnormal liver function				
Yes	51 (65.4)	33 (62.3)	18 (72.0)	
No	20 (25.6)	15 (28.3)	5 (20.0)	
Don't know	7 (9.0)	5 (9.4)	2 (8.0)	
Total	78 (100.0)	53 (100.0)	25 (100)	0.690
Muscle tone and mobility				
Low muscle tone				
Yes	76 (96.2)	51 (94.4)	25 (100.0)	
No	3 (3.8)	3 (5.6)	0 (0.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	0.540
Mobility at best point				
Infant/Not applicable	14 (17.7)	4 (7.4)	10 (40.0)	
Not sitting independently	13 (16.5)	6 (11.1)	7 (28.0)	
Not walking, but sitting independently or crawling	9 (11.4)	7 (13.0)	2 (8.0)	
Walking with support	15 (19.0)	11 (20.4)	4 (16.0)	
Walking independently	28 (35.4)	26 (48.1)	2 (8.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	<0.001
Bone and dental symptoms				
Bone fractures				
One time	20 (25.3)	15 (27.8)	5 (20.0)	
More than 1 time	7 (8.9)	4 (7.4)	3 (12.0)	
No	49 (62.0)	33 (61.1)	16 (64.0)	
Don't know	3 (3.8)	2 (3.7)	1 (4.0)	
Total	79 (100.0)	54 (100.0)	25 (100)	0.839
Dental abnormalities				
Yes	41 (51.9)	34 (63.0)	7 (28.0)	
No	34 (43.0)	17 (31.5)	17 (68.0)	
Don't know	4 (5.1)	3 (5.5)	1 (4.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	**0.009**
Endocrine symptoms				
Adrenal insufficiency or receiving corticosteroid therapy				
Yes	36 (45.6)	26 (48.1)	10 (40.0)	
No	38 (48.1)	26 (48.1)	12 (48.0)	
Don't know	5 (6.3)	2 (3.8)	3 (12.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	0.349
Verbal communication and sensory symptoms				
Verbal communication				
No words	52 (65.8)	29 (53.7)	23 (92.0)	
Less than 50 words	10 (12.7)	9 (16.6)	1 (4.0)	
Yes, greater than 50 words	5 (6.3)	5 (9.3)	0 (0.0)	
Yes, two to three words together correctly (emerging sentences)	2 (2.5)	1 (1.9)	1 (4.0)	
Yes, full sentences	9 (11.4)	9 (16.6)	0 (0.0)	
I don't know	1 (1.3)	1 (1.9)	0 (0.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	**0.021**

Table 1 (*continued*)

Status of patient	Total n (%)	Living n (%)	Deceased n (%)	P-Value
Hearing Loss				
Yes	71 (89.9)	49 (90.7)	22 (88.0)	
No	6 (7.6)	5 (9.3)	1 (4.0)	
Don't know	2 (2.5)	0 (0.0)	2 (8.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	0.084
Hearing change after diagnosis				
Improved	3 (4.2)	1 (2.0)	2 (9.1)	
Worsened	43 (60.6)	34 (69.4)	9 (68.0)	
No change	12 (16.9)	9 (18.4)	3 (4.0)	
Don't know	13 (18.3)	5 (10.2)	8 (36.4)	
Total	71 (100.0)	49 (100.0)	22 (100.0)	**0.021**
Vision impairment				
Yes	70 (88.6)	49 (90.7)	21 (84.0)	
No	4 (5.1)	3 (5.6)	1 (4.0)	
Don't know	5 (6.3)	2 (3.7)	3 (12.0)	
Total	79 (100.0)	54 (100.0)	25 (100.0)	0.363
Cataracts				
Yes	12 (17.1)	9 (18.4)	3 (14.3)	
No	46 (65.8)	34 (69.4)	12 (57.1)	
Don't know	12 (17.1)	6 (12.2)	6 (28.6)	
Total	70 (100.0)	49 (100.0)	21 (100.0)	0.251
Vision change after diagnosis				
Improved	7 (10.0)	5 (10.2)	2 (9.5)	
Worsened	36 (51.4)	29 (59.2)	7 (33.3)	
No change	19 (27.1)	13 (26.5)	6 (28.6)	
Don't know	8 (11.5)	2 (4.1)	6 (28.6)	
Total	70 (100.0)	49 (100.0)	21 (100.0)	**0.022**
Psychological symptoms				
Aggression symptoms				
Yes	14 (17.9)	14 (25.9)	0 (0.0)	
No	61 (78.2)	39 (72.2)	22 (91.7)	
Don't know	3 (3.9)	1 (1.9)	2 (8.3)	
Total	78 (100.0)	54 (100.0)	24 (100.0)	**0.012**
Anxiety symptoms				
Yes	24 (30.8)	20 (37.0)	4 (16.7)	
No	41 (52.5)	24 (44.5)	17 (70.8)	
Don't know	13 (16.7)	10 (18.5)	3 (12.5)	
Total	78 (100.0)	54 (100.0)	24 (100.0)	0.091
Cardiopulmonary symptoms				
Chronic respiratory symptoms				
Yes	22 (28.9)	8 (15.1)	14 (60.9)	
No	53 (69.8)	44 (83.0)	9 (39.1)	
Don't know	1 (1.3)	1 (1.9)	0 (0.0)	
Total	76 (100.0)	53 (100.0)	23 (100.0)	<0.001
Cardiac symptoms				
Yes	8 (10.5)	3 (5.7)	5 (21.7)	

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Table 1 (*continued*)

Status of patient	Total	Living	Deceased	P-Value
	n (%)	n (%)	n (%)	
No	64 (84.2)	48 (90.6)	16 (69.6)	
Don't know	4 (5.3)	2 (3.8)	2 (8.7)	
Total	76 (100.0)	53 (100.0)	23 (100.0)	0.063
Kidney symptoms				
Kidney stones				
Yes	10 (13.2)	7 (13.2)	3 (13.0)	
No	60 (78.9)	44 (83.0)	16 (69.6)	
Don't know	6 (7.9)	2 (3.8)	4 (17.4)	
Total	76 (100.0)	53 (100.0)	23 (100.0)	0.126

Significant differences ($p < 0.05$) in symptom prevalence between living and deceased individuals (chi-squared test) with ZSD are indicated by bolded *p*-value.

their child had 1 seizure, and 37% (n = 20) had experienced more than 1 seizure. Among caregivers of deceased individuals, 72% (n = 18) had responded that their child had more than one seizure throughout their life (p = 0.025 for comparison of seizure occurrence reported between caregiver groups, Table 1). The combined reported prevalence of seizures in all individuals with ZSD was 53%.

When participants were asked about problems with balance, 63% (n = 34) of family caregivers for living individuals with ZSD reported that their child did have balance problems, compared to 32% (n = 8) of caregivers of deceased individuals (p = 0.037, Table 1). Sleep disturbances were reported in 44.4% (n = 24) of living individuals with ZSD, compared to 33% (n=8) of deceased individuals with ZSD.

3.4. Gastrointestinal symptoms

Participants were questioned regarding gastrointestinal and hepatic symptoms in their children. Thirty-five (65%) family caregivers of living individuals with ZSD, compared to all 25 (100%) caregivers of deceased individuals, responded that their child had feeding difficulties (p < 0.001, Table 1). Twenty-two percent (n = 12) of caregivers for living individuals, compared with 60% (n = 15) of caregivers of deceased individuals reported gastroesophageal reflux symptoms in their children (p = 0.003, Table 1).

Abnormal liver function in individuals with ZSD was reported by 62% (n = 33) of family caregivers of living individuals and 72% (n = 18) of caregivers of deceased individuals.

3.5. Muscle tone and mobility

Nearly all participants, including family caregivers of living (94%) and deceased (100%) individuals with ZSD, reported that their child had low muscle tone. When asked about their child's mobility at its best point, 48% of caregivers of living individuals reported that their child walked independently at some point in their life, while only 2% of caregivers of deceased individuals reporting that their child ever walked independently (p < 0.001). Several caregivers of both living (20%) and deceased (16%) individuals reported that their child did walk with mobility support at some point in their life. Nearly half of the caregivers of deceased individuals reported that their child did not live past infancy (40%) and therefore was unable to achieve mobility milestones (Table 1).

3.6. Bone and dental symptoms

Among family caregivers of living individuals with ZSD, 28% (n = 15) of caregivers reported that their child had sustained one bone fracture, and 7% (n = 4) of caregivers reported that their child had 2 or more bone fractures. Among caregivers of deceased individuals, 20% (n = 5) reported that their child had sustained 1 fracture throughout the course of their life, and 12% (n = 3) reported that their child had sustained 2 or more fractures during their life (Table 1).

Among family caregivers of living individuals with ZSD, 63% (n = 34) reported that their child had some dental abnormalities, compared to 29% (n = 7) of caregivers of deceased individuals (p = 0.009, Table 1).

3.7. Endocrine symptoms

Adrenal insufficiency or regular corticosteroid therapy in individuals with ZSD was reported by 48% (n = 26) of family caregivers for living individuals and 40% (n = 10) of family caregivers of deceased children. The combined prevalence of adrenal insufficiency was 45%.

3.8. Verbal communication and sensory symptoms

Fifty-three percent of family caregivers of living individuals with ZSD reported that their child used no words verbally, compared to 92% of caregivers of deceased individuals. The use of full verbal sentences was reported by 16% of caregivers of living individuals, while no caregivers of deceased individuals reported use of full verbal sentences (p = 0.021 for comparison of verbal communication reported by caregiver group). Hearing and vision loss were reported by the majority of caregivers of both living and deceased individuals with ZSD (Table 1). Cataracts were reported by 18% and 14% of caregivers of living and deceased individuals, respectively.

Worsening of hearing and vision over time was reported more frequently by caregivers of living individuals compared to caregivers of deceased individuals (p < 0.023, Table 1).

3.9. Psychological symptoms

Participants were asked about symptoms of aggression and anxiety in their children. Regarding aggression symptoms, 26% (n = 14) of family caregivers for living individuals with ZSD reported aggression symptoms compared to no reports of aggression in caregivers of deceased individuals (p = 0.012, Table 1).

Among family caregivers of living individuals with ZSD, 37% (n = 20), compared to 16.7% (n = 4) of caregivers of deceased individuals, responded observing symptoms of anxiety in their child (Table 1).

3.10. Cardiopulmonary symptoms

When participants were asked about chronic respiratory difficulties in their children, 15% (n = 8) of family caregivers of living individuals with ZSD, compared with 61% (n = 14) of caregivers of deceased individuals reported that their child did have chronic respiratory difficulties (p < 0.0001, Table 1).

When participants were asked about cardiac (heart-related) symptoms, 6% (n = 3) of caregiver for living individuals with ZSD and 21.7% (n = 5) of caregivers of deceased individuals reported their child having cardiac symptoms (Table 1).

3.11. Kidney symptoms

About 13% of all family caregivers responded that their child had kidney stones (Table 1).

4. Discussion

In the present study, we report a comprehensive account of the prevalence of various symptoms and related assessments in ZSD using family caregiver observation and input. As a spectrum disorder, ZSD

presents with a broad variety of symptoms which makes it challenging for health care providers to estimate disease severity and prognosis. Additionally, although some studies have associated specific *PEX1* gene variants with milder or more severe forms of ZSD, there is no consistent evidence of strict genotype-phenotype correlations in ZSD [6,29]. Our study provides a unique and novel tool to face this challenge by using the caregivers' *perception* of disease severity, thus complementing traditional clinical assessments. For example, we observed that more family caregivers of deceased individuals with ZSD reported that they considered their child's disorder as "severe", compared to caregivers of living individuals. This finding is somewhat expected because the prevalence of many symptoms (neurological, gastrointestinal and sensory symptoms, as well as symptoms related to communication and mobility) in deceased individuals diagnosed with ZSD of the study is greater than in living individuals, as reported by their caregivers. We expect that grouping "severe" cases from other cases would show a significantly greater occurrence of symptoms in severe cases versus cases that were not considered severe. Based on these data, this study points to *perception* of severity by caregivers as a unique variable that encompasses all symptoms predicting clinical severity. Although a subjective variable, this could be used as a surrogate for clinical assessment of disease severity and inform on long-term prognosis.

Regarding the increased prevalence of dental symptoms reported by caregivers of living children with ZSD, previous case studies report that dental abnormalities in ZSD occur upon eruption of adult teeth [12,30]. It is likely that the deceased individuals with ZSD in this study, who had a median survival of 1.2 years, had passed away prior to eruption of adult teeth. Other symptoms, such as verbal communication and mobility, may also show differences due to the median age between study groups. Future studies using newly available tools to assess severity indices among children with ZSD [31] may help further characterize symptom presentation in mild to intermediate ZSD from more severely affected populations.

Our findings of low muscle tone, abnormal liver function, and the occurrence of vision and hearing loss are consistent with previous clinician-led studies [6,7,9], suggesting comparable accuracy of data between clinician reports and caregiver-reported outcomes. Our study also found seizures occurred in 72% of the participants with deceased children, and 44% of participants with living children. An earlier study reported seizures in 7 of 9 primarily severely affected ZSD patients [20]. This is consistent with our data from participants with deceased children and points again to the validity of caregiver estimates of disease severity. A study in older ZSD patients with a milder phenotype found that seizures occurred in 24% of the 31 study subjects [6], i.e. half of the prevalence of seizures reported by our study participants with living children. Considering our larger sample size, our findings may be closer to indicating the real prevalence of seizures in the ZSD population. Actually, seizure prevalence may be even higher than 44%, because not all participants reported that their child ever had an EEG, thus raising the possibility that seizures may have been overlooked by caregiver observation.

We also found that adrenal insufficiency occurred in 48% of living children with ZSD. This finding is nearly double the prevalence reported in a previous study of 24 ZSD patients with a milder phenotype [10]. Again, considering the larger sample size in our study and our broader phenotype spectrum, we believe that our report may be a more accurate reflection of the prevalence of adrenal insufficiency in the ZSD population.

About a third of our participants (caregivers of both living and deceased individuals) reported bone fractures in their children. One study reporting low bone mineral density in ZSD patients found that 4 of the 13 patients studied had sustained bone fractures throughout the course of their life [8]. Our findings align with and expands on this earlier study, showing a similar prevalence in a larger representative sample of individuals with ZSD. Previous publications have recommended periodic monitoring of bone mineral density with dual x-ray absorptiometry (DXA) [4,8]. About a quarter of our participants have reported abnormal DXA results in their children, further emphasizing the need for consistent monitoring of bone density in individuals with ZSD.

4.1. Study implications

Our study has multiple implications. First, this study highlights the value of the family caregiver in being able to report on symptoms in ZSD; this may be useful and provide less of a barrier in collecting larger-scale data in ZSD and other rare diseases, compared to data collected in a more conventional clinical setting. We were able to systematically survey a more comprehensive range of ZSD symptoms compared to previous studies by including the prevalence of communication issues, mobility symptoms, dental symptoms, bone fractures, neuropsychological symptoms, gastroesophageal reflux, respiratory symptoms and cardiac symptoms in ZSD. Taken together, these new findings emphasize the considerable burden of ZSD on patients and their families, and the need for targeted clinical care of patients affected by ZSD. This information may also help guide efforts in the development of appropriate outcome measures to monitor in future natural history studies and clinical trials for ZSD. Preclinical studies have identified several flavonoid molecules as potential therapeutic candidates [32,33] and current advocacy efforts in ZSD are supporting preliminary studies in gene therapy for ZSD. Taken together, this study as well as other current research will be important steps in improving health outcomes for patients with ZSD.

Our results suggest previous studies may have underestimated the prevalence of seizures and adrenal insufficiency in ZSD, perhaps highlighting a need for closer monitoring and evaluation of seizures and adrenal function. Moreover, as the majority of our participants reported both hearing and vision loss in their children, the impact of the combined dual sensory loss in ZSD on communication and daily functioning requires more extensive characterization as opposed to addressing each symptom individually. Given the significant burden and necessary management of these symptoms, this study suggests that there is a need for adequate resources and support for family members caring for a patient diagnosed with ZSD. Future studies confirming our findings will be important in ensuring that medical professionals do not underestimate the impact of ZSD when managing medical care for their patients.

4.2. Study limitations and future studies

The present study is a cross-sectional survey that relied solely on caregiver observation and report. Although our findings were consistent with those of several clinician-led studies measuring similar outcomes in ZSD patients, it is important to note that family caregivers are not formally trained to recognize clinical signs and symptoms, which may result in inaccuracies in the data. To address this, we developed our survey instrument with pilot data from caregivers' input regarding survey items and associated responses to minimize comprehension issues. These efforts resulted in the inclusion of the "I don't know" item response in several survey question items. While this response was the least selected item for most survey questions, there were a considerable number of participants who selected "I don't know" when asked about balance issues, sleep disturbances, reflux and liver abnormalities in their children (Table 1), suggesting the need for further refinement of wording for these items in future surveys.

The majority of our participants identified as mothers of individuals with ZSD and reported their race as white. Although this is common in rare disease research [34], future studies will need to adopt more targeted recruitment strategies to ensure that participants from underrepresented demographic categories are included in these studies.

Another novel aspect of our research was the inclusion of caregivers of both living and deceased children with ZSD. The amount of time to recall symptoms in their child is inherently different between these two

groups of participants, therefore differences between groups may be at least partially attributable to lapse in recall. However, most caregivers of children' with ZSD are highly engaged in their child's medical care and the finding that the frequency of some symptoms tended to be relatively greater in the deceased patients would suggest that caregiver recall was not significantly compromised despite the study design. By utilizing the input of bereaved caregivers for children with ZSD we were able to enroll a larger number of participants and describe a broader characterization of the ZSD patient population compared to previous clinician-led studies focusing on living patients alone. Future studies will need to explore approaches to better incorporate data from deceased patients, as well as account for differences in age between groups at the time of study, into clinical research on ZSD.

Considering that nearly all of the participants were parents of patients with ZSD, it is possible that there may be some attentional bias and subjectivity in the data when parents were asked to report on their child's condition. To address this, we limited the questions in our symptom inventory to primarily observable and less subjective concepts. For future studies of rare diseases like ZSD, a combination of clinician-led assessment together with caregiver report will better confirm robustness of the clinical data as well as provide a richer characterization of the true phenotypic variation.

5. Conclusions

To our knowledge, this is the largest study reporting on the prevalence of symptoms in ZSD. We included several symptoms seldom addressed in previous studies of ZSD patients, such as dental abnormalities, balance issues, sleep disturbance, and psychosocial symptoms. By leveraging input from family caregivers of both living and deceased children, we defined a broad perspective of patient features. This novel approach to data collection should be useful in conjunction with clinician-led studies for defining the ZSD phenotype, targeting clinical management, informing future therapeutic trials and predicting prognosis.

Acknowledgements

The authors would like to thank the study participants, as well as the Global Foundation for Peroxisomal Disorders for the opportunity to collect pilot data at their family conference.

Funding

This study was supported by a Training and Career Development Award to MB (10 U54 DK 83916) from the Rare Disease Clinical Research Network, and the Sterol and Isoprenoid Research Consortium to WBR (U54 HD061939) of the National Center for Advancing Translational Sciences and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, and NIH.

Declaration of Competing Interest

Authors have no relevant conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2020.100694.

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