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ABSTRACT

Kleefstra syndrome (KLEFS1) is a rare genetic neurodevelopmental disorder affecting multiple body systems. It continues to be under-researched, and its prevalence remains unknown. This paper builds on the international KLEFS1 cohort of 172 individuals based on the caregiver-reported outcomes collected within the online data collection platform GenIDA and reports the occurrence, frequency and severity of symptoms in KLEFS1. The study clearly shows the importance of caregiver-reported outcomes collections in the rare disease domain. Moreover, the study emphasizes the need for more specific and enhanced data collection methods, suggesting recommendations to optimize caregiver-reported registries and foster an even more profound understanding of rare diseases.

1. Introduction

In the realm of rare diseases, dedicated data collection platforms for patient-reported outcomes (PROs) or caregiver-reported outcomes (CROs) (sometimes called observer-reported outcome assessments) are of major importance (Benjamin et al., 2017; Kölker et al., 2022). In contrast to common diseases where medical professionals possess extensive knowledge and clinical data, in rare diseases the medical and research community benefits from the first-hand expertise of patients, parents, and caregivers who possess invaluable insights. In rare disease research, traditional clinic-based natural history studies face limitations due to low patient enrolment, irregular study visits attendance, and logistical challenges. This can hinder the collection of comprehensive data for disease management and clinical trial design. Using online self-reported data offers a valuable alternative, enabling the gathering of symptom and treatment information with greater participation ease compared to clinician-led studies (Bose et al., 2020). These specialized data collection platforms are tailored to the unique requirements of rare diseases. For instance, they must possess a global reach since the patient cohorts for rare diseases are often exceedingly limited. Additionally, offering accessibility in various languages is preferable to facilitate broader and more inclusive participation.

In this study, our primary data source is GenIDA *(Genetics of Intellectual Disability and Autism Spectrum Disorders)*,[1] an international online data collection platform which main objective is to gather information on the natural history, medical complications, behavioural disorders and responses to pharmacological treatments for patients affected by intellectual disabilities (ID) and autism spectrum disorder (ASD) of genetic origin. GenIDA aims to improve the understanding and management of these disorders based on the information provided by

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[1] Platform available at https://genida.unistra.fr/.

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caregivers. GenIDA has both caregivers and professionals registered, the former are given the possibility to report about the symptoms and the latter to use GenIDA as an information point for specific rare diseases. A total of 1417 caregivers across 35 genetic conditions are labelled as active GenIDA participants. GenIDA data collection is based on 46 in-depth multi-level questions (Burger et al., 2023).

In this paper, our focus lies on Kleefstra syndrome (KLEFS1; OMIM #61025[2]), a rare monogenetic syndrome, caused by haploinsufficiency of the euchromatic histone methyltransferase 1 (*EHMT1*) gene, an important regulator of the epigenetic machinery. The clinical features include ID, autistic-like features, childhood hypotonia, and distinctive facial features. The majority of individuals function in the moderate-to-severe spectrum of ID although a few individuals have mild delay. While most have severe expressive speech delay with little speech development, general language development is usually at a higher level, making nonverbal communication possible. A complex pattern of other findings can also be observed; these include heart defects, renal/urologic defects, genital defects in males, severe respiratory infections, epilepsy/febrile seizures, psychiatric disorders, and extreme apathy or catatonic-like features after puberty (Bloemendaal et al., 2023; Kleefstra and de Leeuw, 1993/2023).

There have been only a few published studies involving KLEFS1 cohort sizes (Bouman et al., 2024; Morison et al., 2024; Willemsen et al., 2012). This paper presents the largest caregiver-reported study to date on KLEFS1 symptom prevalence, showing the occurrence, frequency and severity of KLEFS1 symptoms.

2. Data and methods

KLEFS1 is a complex disorder, involving many body systems. The GenIDA questionnaire was designed to collect the clinical spectrum of an individual and focuses on a wide scale of clinical symptoms.

2.1. Data collection

GenIDA data collection has been ongoing since 2016, and our analysis encompasses the information gathered during the period spanning from 2016 to 2022. Study participants provided their survey responses through online forms, either in a single session or through multiple sessions. Our analysis considered all survey responses, including those that were partially completed.

2.2. Data preparation and statistical analyses

The data was exported in a raw data format, more precise as PDF files where each file corresponded to a single user/individual. We extracted and organized the file's content using custom-developed PDF parsers. Once the data was extracted and organized, we analysed it using data science techniques. For this, we used Pandas,[3] an open-source data analysis software built on top of the Python programming language. Basic summary statistics techniques were used. Prevalence is reported as the ratio of yes answers in relation to the total number of answers per specified symptom.

3. Results

3.1. Demographics

Reviewing the demographics, the KLEFS1 cohort includes 172 individuals (N = 172), respectively 82 males (47.7%), and 90 females (52.3%); living in the USA, UK, France, Germany, Australia, Spain, The Netherlands, Canada, Switzerland, Brazil, Italy, Colombia, Slovenia, Denmark, Portugal, Hungary, Japan, Egypt, Luxembourg, Ireland, Albania, India, Belgium, South Africa, New Zealand, Norway, Greece and Israel. The mean age is 12.92 years (range 1–45 years, median 10 years).

3.2. Overview

The aggregated results are first presented in the form of an overview in the main figure (Fig. 1), introducing 25 specific clinical symptoms/traits and their prevalence in the KLEFS1 cohort: *Behaviour problems, Vision problems, Problems during newborn period (first four weeks), Walking problems, Diagnosis of intellectual disability, Problems during pregnancy/labour/delivery, Musculo-skeletal problems, Digestive problems, Feeding problems, Sleeping problems, Dental anomalies, Skin, nails and hair problems, Oral/Buccal problems, Cardiac problems, Other mouvement disorders, Hearing problems, ASD, Respiratory and pulmonary problems, Renal, bladder and urogenital problems, Epilepsy, Endocrine and metabolic problems, Tremor, Vascular problems, Sense of smell problems, Cancer development*. The order of the clinical symptoms is important, showing first the most frequently observed symptoms, followed by the less frequent ones (lower prevalence). Also, Fig. 1 shows the affected (yes/positive answers) and unaffected (no/negative answers) individuals and shows when the answer was unknown. If available, the severity bar chart is included. The majority of observed clinical symptoms (20/25) are frequently reported in KLEFS1 (>25%). The top five reported clinical symptoms include behaviour problems (67.1%), vision problems (66.9%), problems during the newborn period (66.5%), walking problems (65.6%) and diagnosis of ID (64.7%). Subsequently, an in-depth interpretation of the results for each clinical symptom is presented in the chapters 3.3 to 3.27.

3.3. Behaviour problems

From 158 caregivers who provided answers for this trait, 106 (67.1%) confirmed the presence of behaviour problems, 44 (27.8%) denied them and 8 (5.1%) answered with "*I don't know*". Those, who confirmed behaviour problems, selected which specific behaviour problems were present and indicated the level of severity (*mild, moderate, major*). The most reported behaviour problems are repetitive behaviour, attention deficit, anxiety, impulsivity, restricted interests, aggressive behaviour and obsessions. Observed behaviour problems include also aggressive behaviour, hyperactivity, phobias, shyness and depressive tendencies, whereas diagnosed schizophrenia is generally not present (prevalence 1.3%). Details with trait specific prevalence are presented in Fig. 2.

3.4. Vision problems

In sum, 166 caregivers participated and of these 111 confirmed vision problems (66.9%), while 42 (25.3%) reported that vision problems were absent and 13 (7.8%) answered with "*I don't know*". Those, who confirmed vision problems, stated that the major problem is hypermetropia (problems with near vision, *far-sighted*), with a prevalence of 39.2%. Moreover, 23.5% stated *squint/cross eyed* (*strabismus*). Specific vision problems with their prevalence are shown in Fig. 3. Additionally, in relation to vision problems, KLEFS1 individuals often wear glasses, namely in 51.2%.

3.5. Problems during newborn period (first four weeks)

The majority (109 individuals representing 66.5%) of KLEFS1 individuals experienced health problems in the newborn period (first four weeks). 51 caregivers (31.1%) stated no problems, and 4 (2.4%) didn't know how to answer. Frequently reported newborn period problems (Fig. 4) are *feeding difficulties* (72 answers/43.9%), *hypotonia at birth* (62 answers/37.8%) and *jaundice* (48 answers/29.3%), while 35 caregivers

[2] https://omim.org/entry/610253
[3] https://pandas.pydata.org/.

Fig. 1. Overview of groups of clinical symptoms in the KLEFS1 cohort with prevalence numbers. The majority of observed clinical symptoms (20/25) are frequently reported (from epilepsy to behaviour problems) ranging within the prevalence of 25% to high 67.1%. Answers from caregivers are presented as a bar chart with sample size (n). If applicable, the severity is presented on a scale of 3 stages (*mild, moderate, major*), while two groups of clinical symptoms (*Intellectual disability* and *Hearing problems*) use 4 stages in reporting (namely *mild, moderate, severe, profound*).

(21.3%) reported other problems that were not listed.

On the additional question, asking what treatment was performed in relation to problems during newborn period, 37 answers (57.8%) reported *tube feeding* and 27 answers (42.2 %) reported *assisted ventilation* (Fig. 5).

3.6. Walking problems

Walking problems are identified in 103 individuals (65.6%), 47 caregivers didn't indicate walking problems (29.9%), 7 stated that they don't know (4.5%). The major specified walking problem is *unstable walk*, reported by 50 caregivers (31.8%). Details are presented in Fig. 6. The reported seriousness is more or less equally distributed (based on evaluation of seriousness of walking problems from caregivers): mild (35.3%), moderate (32.4%), major (32.4%).

3.7. Diagnosis of intellectual disability (ID)

KLEFS1 is known to be associated with ID. 108 caregivers confirmed that their child has a diagnosis of ID (64.7%), 27 answered that their children with KLEFS1 don't have a diagnosis of ID (16.2%) and 32 answered with I don't know (19.2%). The distribution of the degree of ID shows a higher number of those having moderate (31 answers/30.1%) and severe (51 answers/49.5%) ID. Only 7 caregivers (6.8%) reported profound ID, whereas 14 caregivers (13.6%) reported mild ID. In addition, the caregivers were asked if an IQ test was performed, but the majority of KLEFS1 cohort did not undergo an IQ test according to caregivers (110/160, 68.8%), only 37 caregivers reported that an IQ test has been performed (23.1%) and 13 didn't know (8.1%).

3.8. Problems during pregnancy/labour/delivery

Our data indicates that problems during pregnancy, labour and delivery (Fig. 7) are important, as 108 caregivers (62.8%) reported them as present, 56 (32.6%) as negative and 8 (4.7%) with I don't know. Most frequently reported is the *caesarean section* (26.2 %) followed by *excessive amniotic fluid* (16.9%). In addition, the reported Apgar scores indicate the lowest Apgar scores at 1 min (mean 7.78) and higher Apgar scores at 5 min (mean 9).

3.9. Musculoskeletal problems

Data shows that 90 individuals (57.3%) indicated musculo-skeletal problems, 54 responded with no problems (34.4%) and 13 did not know (8.3%). Detailed specification reveals that the most frequent symptom is *joint laxity (ligamentous laxity,* looseness, 21%), followed by *pes planus (flat feet*, 19.7%), *scoliosis* (11.5%) and *skull anomaly or malformation* (10.2%) (Fig. 8).

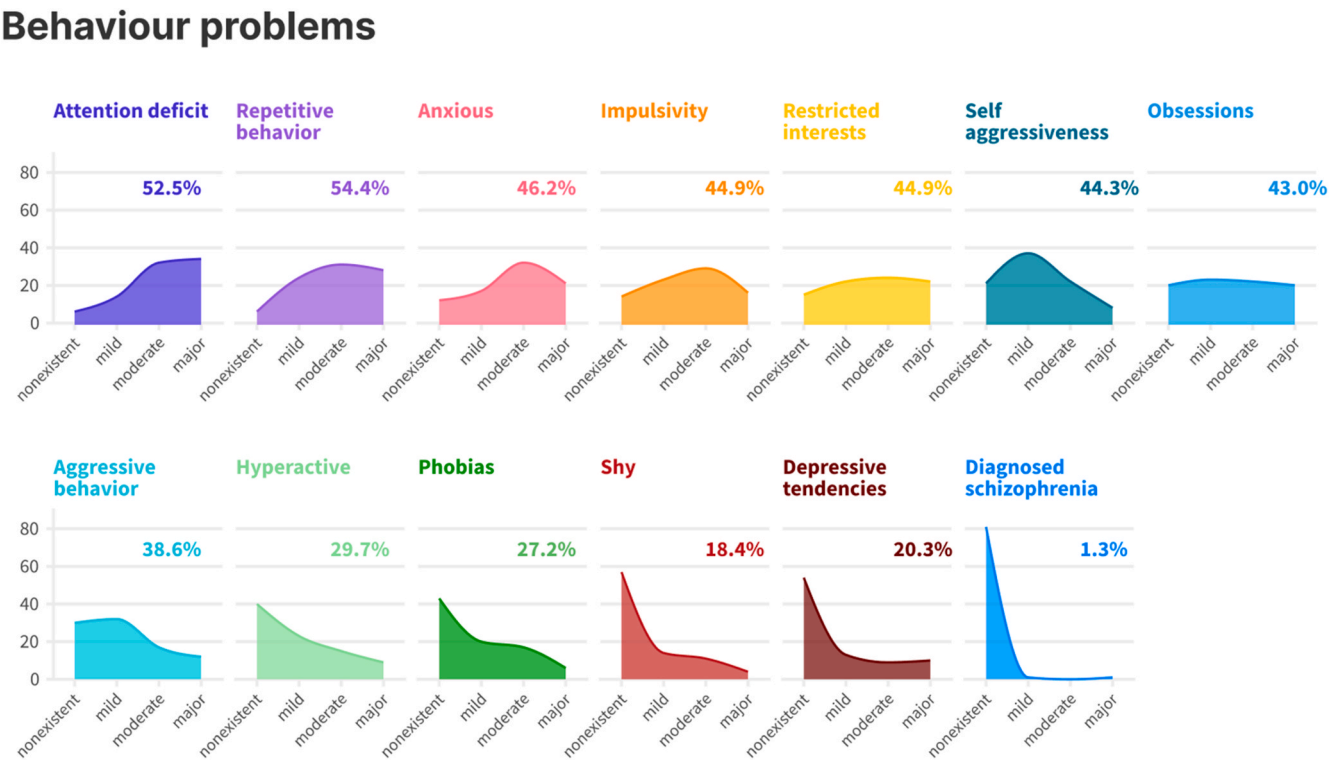

Fig. 2. Specific behaviour problems with levels of severity and prevalence. Densities represent distribution of answers for specific behaviour problems. Percentages depict prevalence (e.g., Attention deficit: prevalence is 52.5%).

Vision problems	Prevalence [%]	YES /NO / I don't know	n=
Far-sighted	39.2		79
Squint/cross eyed (strabismus)	23.5		68
Short-sighted	10.2		63
Nystagmus (involuntary eye movement)	6		54
Cataract	0.6		51

Fig. 3. Specific vision problems.

Problems during newborn period-first 4 weeks	Prevalence [%]	YES /NO	n=
Feeding difficulties	43.9		109
Hypotonia at birth (muscle weakness)	37.8		109
Jaundice	29.3		109
Signs of anoxia (lack of oxygen)	19.5		109
Hypertonia at birth	1.8		109
Other	21.3		109

Fig. 4. Problems during newborn period.

Treatments during newborn period-first 4 weeks	YES /NO	n=
Tube feeding		64
Assisted ventilation		64
Other		64
Gastrostomy (tube connecting the stomach)		64
Jejunostomy (tube connecting the small intestine)		64

Fig. 5. Treatments during newborn period.

Walking problems	Prevalence [%]	YES /NO	n=
Unstable walk	31.8		103
Walk for a limited period, on a limited distance	12.7		103
Never learned to walk	12.1		103
Walk with cane or other aid	4.5		103
Learned to walk but stopped walking	1.3		103
Other	19.7		103

Fig. 6. Walking problems.

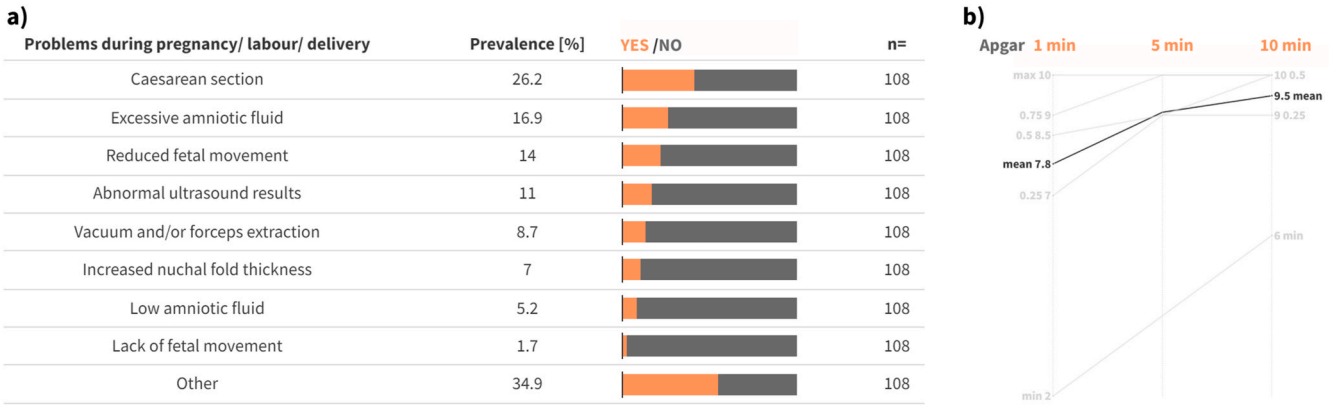

Fig. 7. Problems during pregnancy/labour/delivery a) Showing specific problems during pregnancy/labour/delivery with prevalence and yes/no answers. b) Apgar scores with mean values.

3.10. Digestive problems

Digestive problems were indicated in 97 individuals (57.1%), 60 individuals stated no problems (35.3%) and 13 were not sure (7.6%). Those that indicated digestive problems, specified them most often as *constipation* (29.4%), followed by *regurgitation* (15.9%) and *repeated vomiting episodes* (14.1%). The reported constipation was mostly reported as major (41.5%). Fig. 9 shows distributions of severity of digestive problems in detail, together with the prevalence.

3.11. Feeding problems

Feeding problems were indicated from 85 caregivers (52.1%), denied from 76 individuals (46.6%), following 2 individuals being unsure (1.2%). Specification of feeding problems is presented in Fig. 10, showing that the most reported problem is *eats too much* (16%), other are less frequently reported (*craves for food* with a prevalence of 13.5%, *eating only very restricted food* with a prevalence of 10.4% and *does not want to eat (anorexia)* being almost non-existent), but also many caregivers reported other (27.6%). However, the seriousness of feeding problems was evaluated as significant (major: 40.7%, moderate: 43.2% and mild: 16%).

Musculo- skeletal problems	Prevalence [%]	YES /NO / I don't know	n=
Joint laxity (ligamentous laxity, looseness)	21		49
Pes planus (flat feet)	19.7		47
Scoliosis	11.5		45
Skull anomaly or malformation	10.2		45
Pectus excavatum (hollowed chest)	7.6		44
Malformation of the vertebral column	5.1		39
Hip dysplasia (deformation or misalignment of the hip joint)	3.8		38
Contractures (permanent shortening of a muscle or joint)	3.2		39
Club foot (congenital talipes equinovarus CTEV)	3.2		37
Kyphosis (Hunchback/hump back)	3.2		40
Malformation of the lower limb	3.2		38
Pes cavum (a high arch of the feet)	1.9		35
Fragility (ex:broken bones)	1.9		35
Malformation of the upper limb	1.3		38
Pectus carinatum (pigeon chest)	1.3		37

Fig. 8. Musculoskeletal problems.

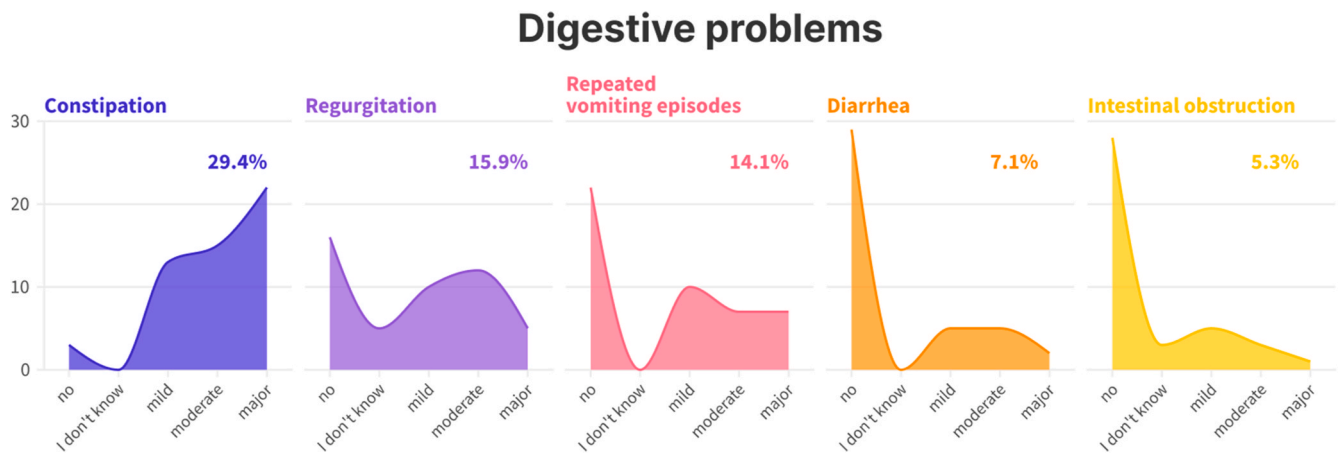

Fig. 9. Digestive problems with levels of severity and prevalence. Densities represent distribution of answers for specific digestive problems. Percentages depict prevalence (e.g., Constipation: prevalence is 29.4%).

3.12. Sleeping disorders

Sleeping disorders are present in 84 individuals, representing 50% of the cohort. 71 individuals don't have sleeping disorders (42.3%) and for 13 individuals caregivers were not sure (7.7%). Level of seriousness was mostly evaluated as major (40.5%) and moderate (39.2%), followed by mild (20.3%).

3.13. Dental anomalies

Dental problems were reported by 75 caregivers (47.8%), 56 caregivers stated that dental problems are not present (35.7%) and 26 were not sure (16.6%). Dental anomalies are presented in Fig. 11, showing that most caregivers have reported *complex orthodontics* (14%) and *too few teeth* (11.5%) as prevalent dental anomalies. However, 44 caregivers (28%) reported other problems that were not listed. Seriousness of dental problems was mostly evaluated as mild and moderate (each 43.8%), while there were also major dental anomalies reported, but only in 12.3%.

3.14. Skin, nails and hair problems

Skin, nails and hair problems were reported by 69 caregivers (44.8%), 81 indicated no problems (52.6%), 4 were not sure (2.6%). The most reported problems (Fig. 12) include *abnormal nails (soft, brittle, unusual shape, etc.)* (22.7%), *eczema, psoriasis or other skin inflammatory*

Feeding problems	Prevalence [%]	YES /NO	n=
Eats too much	16		85
Craves for food	13.5		85
Eats only very restricted food	10.4		85
Does not want to eat (anorexia)	0.6		85
Other	27.6		85

Fig. 10. Feeding problems.

Dental problems	Prevalence [%]	YES /NO	n=
Complex orthodontics	14		75
Too few teeth	11.5		75
Frequent dental caries	8.9		75
Too many teeth	1.9		75
Other	28		75

Fig. 11. Dental anomalies.

Skin, nails and hair problems	Prevalence [%]	YES / NO	n=
Abnormal nails (soft, brittle, unusual shape, etc.)	22.7		70
Eczema, psoriasis or other skin inflammatory problems	17.5		70
Fine wispy hair (unlike parents or siblings)	13.6		70
Hair color different from parents and siblings	3.9		70
Skin pigmentation disorder (e.g. hypopigmentation)	2.6		70
Thick hair (unlike parents or siblings)	1.9		70
High number of moles	0.6		70
Noninflammatory anomalies of the skin (scar, photosensibility, etc.)	0.6		70
Other	8.4		70

Fig. 12. Skin, nails and hair problems.

problems (17.5%) and *fine wispy hair (unlike parents or siblings)* (13.6%). The majority of caregivers stated that these problems are mild (82.4%), 14.7% have evaluated them as moderate and 2.9% as major.

3.15. Oral/buccal problems

Oral/buccal problems were reported from 63 caregivers (41.2%), 69 stated no problems (45.1%), 21 were not sure (13.7%). Fig. 13 shows specific problems, *hypersalivation* (prevalence of 20.9%) and *swallowing difficulties* (prevalence of 19.6%) being most frequently reported. Regarding the seriousness, these problems were mostly reported as moderate (44.1%) and mild (39%), followed by major (16.9%).

3.16. Cardiac problems

Cardiac problems were reported from 65 caregivers (39.9%), however 84 indicated no problems (51.5%) and 14 were not sure (8.6%). The most frequent reported cardiac problems are *atrial septal defect (ASD)* (11.7%) and *ventricular septal defect (VSD)* (9.2%), followed by *pulmonary stenosis* (8.6%). Other cardiac problems (see Fig. 14) are less frequently observed in KLEFS1 individuals.

3.17. Other movement disorders

Movement disorders were reported by 64 caregivers (39.8%), 82 did not report them (50.9%), 15 caregivers being unsure (9.3%). Fig. 15

Oral/Buccal problems	Prevalence [%]	YES / NO	n=
Hypersalivation	20.9		62
Swallowing difficulties	19.6		62
Cleft lip	0.7		62
Cleft palate	0.7		62
Other	20.9		62

Fig. 13. Oral/buccal problems.

Cardiac problems	Prevalence [%]	YES /NO / I don't know	n=
Atrial septal defect (ASD)	11.7		34
Ventricular septal defect (VSD)	9.2		38
Pulmonary stenosis	8.6		31
Cardiac or cardiovascular malformation	6.1		31
Cardiac rythms problems (abnormal EKG)	5.5		32
Patent ductus arteriosus (PDA)	5.5		30
Bicuspid aortic valves (BAV)	4.3		25
Cardiomyopathy	0.6		26
Tetralogy of Fallot (TOF)	0		25
Aortic aneurysm (dilatation/ broadening)	0		21

Fig. 14. Cardiac problems.

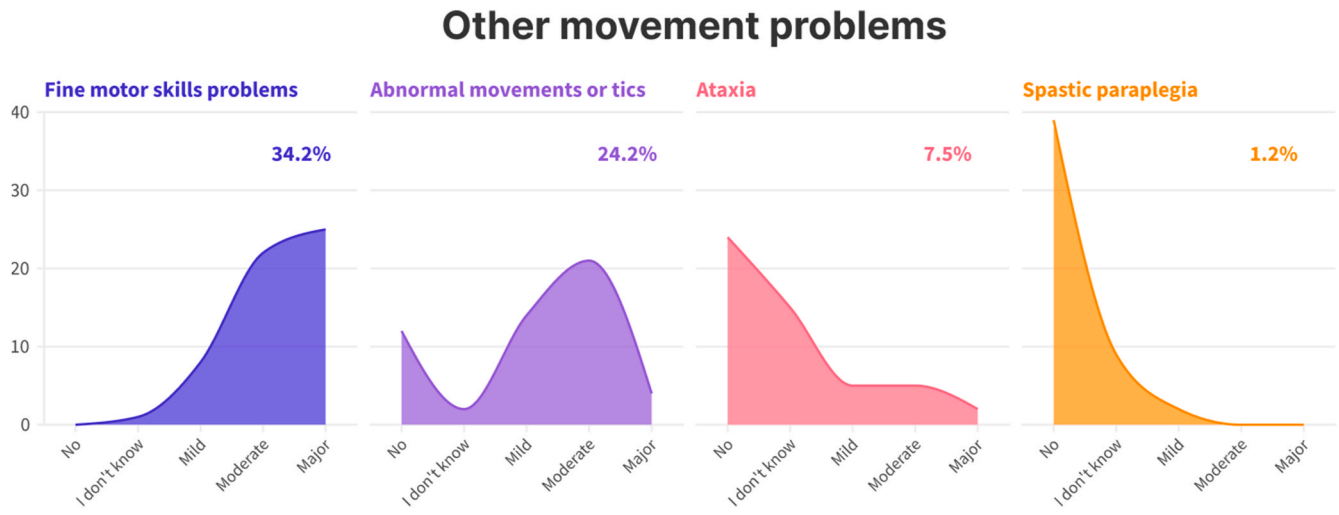

Fig. 15. Other movement disorders with levels of severity and prevalence. Densities represent distribution of answers for specific problems. Percentages depict prevalence (e.g., Fine motor skills problems: prevalence is 34.2%).

represents the distribution of answers for specific disorders of movement. 34.2% of individuals have *fine motor skills problems*, being more or less evaluated as major or moderate. Next, *abnormal movements or tics* are reported with a prevalence number of 24.2% (mostly moderate seriousness), followed by *ataxia* (prevalence 7.5%) and being mostly moderate and mild.

3.18. Hearing problems

Hearing problems were reported from 66 caregivers (39.5%), 88 stated no problems (52.7%), 13 were not sure (7.8%). Fig. 16 shows the stated hearing problems. *Recurrent ears infections* are the most frequently reported problem (20.4%), followed by *deafness* (9%) and *ear malformation* (3.6%). Moreover, caregivers were also asked about the seriousness of the hearing impairment and only 15 caregivers were taking part in answering (n = 15), showing that hearing problems are more or less severe (46.7%) or mild (33.3%). Moderate problems were stated in 13.3% and profound in 6.7%. The following statements were used for seriousness evaluation: *Mild - troubles in noisy situations. Moderate - can hear and understand speech only in quiet situations. Severe - cannot hear a speech without the use of amplification. Profound - cannot hear most environmental sounds without the use of amplification.*

Also, the caregivers who stated that their children have hearing problems, were asked how are the hearing problems managed (Fig. 17) and KLEFS1 individuals do use *hearing aids* quite often (34.8%), even more have *hearing tubes (ear or tympanostomy tubes)* (39.4%).

3.19. Autism spectrum disorder

Autism spectrum disorder (ASD) was reported from 45 caregivers (27.1%), 97 caregivers (58.4%) didn't report ASD and 24 caregivers (14.5%) were not sure. Regarding the autism test, 39 caregivers (23.5%) reported that an autism test has been performed, 110 caregivers (66.3%) responded with no and 13 caregivers (7.8%) didn't know.

3.20. Respiratory and pulmonary problems

Respiratory and pulmonary problems were reported from 41 caregivers, resulting in a prevalence number of 26.3%. The majority of caregivers (104) didn't report respiratory and pulmonary problems (66.7%), 11 caregivers (7.1%) did not know. The seriousness of these problems was rated as mostly moderate (52.8%), mild (25%) and major (22.2%). However, the prevalence for these problems (*asthma, hay fever, respiratory rate problem* etc.) were low ranging up to 5.1% - this being the highest prevalence namely for *asthma* (see Fig. 18).

3.21. Renal, bladder and urogenital problems

Renal, bladder and urogenital problems were reported from 39 caregivers (25.2%), the majority didn't report problems (98 caregivers representing 63.2%), 18 caregivers were not sure (11.6%). Fig. 19 shows a specification of problems, all ranging with a low prevalence number from 3.9% to 5.2%. *Renal malformation (*e.g.*, horseshoe kidney)* and *urinary system malformation* occur with a prevalence of 5.2%, while *frequent urinary tract infection* and *undescended testicles (cryptorchidism)* occur with a prevalence of 4.5%.

3.22. Epilepsy

In relation to epilepsy, 39 caregivers reported that KLEFS1 individuals had seizures, resulting in a 25% prevalence. The majority of caregivers, 107 (68.6%), didn't report seizures, while 10 (6.4%) didn't know. The most reported types of seizures were *absence seizures* and *grand mal seizures (tonic-clonic seizures)* with a prevalence of 9% alongside with *febrile convulsions (fever fit)* and *complex partial seizures (focal seizures)* with a prevalence of 6.4% (Fig. 20).[4]

All caregivers reporting seizures were asked if the epilepsy requires constant medication and 20 (66.7%) said yes, 10 (33.3%) said no (n = 30). Caregivers also stated specific epilepsy medication used (*name of the drug/molecule*): Valproic Acid, Levetiracetam, Lamotrigine, Diazepam, Lacosamide, Carbamazapine, Cannabidiol, Rufinamide, Vigabatrin, Clonazepam, Clobazam, Topiramate, Zonesamide. In addition, also VNS (*vagus nerve stimulation*) therapy was stated to be used. Subsequently, caregivers were asked about the effectiveness of the drug. 13 caregivers (50%) reported *good* effectiveness of the drugs, 10 caregivers (38.5%) reported *very good* effectiveness, 1 caregiver (3.8%) reported *little effect* and 2/26 caregivers (7.7%) have chosen *other* as their answer. Besides that, caregivers were also asked if the epilepsy is pharmaco-resistant, and the majority (26 caregivers or 86.7%) stated no, while 4/30 caregivers (13.3%) stated yes.

3.23. Endocrine and metabolic problems

Endocrine and metabolic problems were reported from 22 caregivers resulting in a prevalence of 14.3%. 93 caregivers (60.4%) didn't report these problems, while a high number of caregivers (39 or 25.3%) was not sure, that is why the answer *I don't know* was chosen. Fig. 21 shows the specific endocrine and metabolic problems, with *hypothyroidism (under-active thyroid)* as the most frequent, with a prevalence of 7.1%. Caregivers were also asked about the age at first menstruation (if applicable), and 16 caregivers responded, resulting in a mean age of 11.8 years (range 8–15 years) for first menstruation.

3.24. Tremor

Caregivers reported if KLEFS1 individuals have tremors. Out of 161 caregivers, 22 reported tremors resulting in a prevalence of 13.7%, 132 (82%) didn't report tremors and 7 (4.3%) didn't know. The seriousness of tremors shows that most of them are evaluated as mild (59.1%), 31.8% as moderate and 9.1% as major.

3.25. Vascular problems

In relation to vascular problems, 13 caregivers reported them, occurring in a prevalence of 8.4%. In 70.1% cases vascular problems were not reported and in 21.4% caregivers didn't know. *Hypertension* with a prevalence of 1.3% was most often reported (Fig. 22).

3.26. Sense of smell problems

Caregivers were also asked about smell problems and only 4 responded with yes, resulting in a prevalence of 2.7%. 43.3% of caregivers of KLEFS1 individuals didn't state smell problems, but the majority (54%) were not sure.

3.27. Cancer development

Only 2 caregivers reported cancer, resulting in a prevalence of 1.3%. 135 caregivers (90%) responded with no cancer development, while 13 caregivers (8.7%) were not sure. One patient received a diagnosis of rhabdomyosarcoma at the age of 12 years, while the other was diagnosed during the pre-stadium phase of oesophageal cancer, potentially linked to erosions caused by gastroesophageal reflux disease.

4. Discussion

The present study contributes valuable insights into the clinical spectrum and prevalence of symptoms associated with KLEFS1. This research builds upon an international cohort of 172 individuals, making it the most extensive caregiver-reported study to date on KLEFS1 symptomatology.

The utilization of CROs (PROs) is proved to be crucial in addressing the challenges faced by traditional clinic-based studies in rare diseases (Benjamin et al., 2017; Ciani and Federici, 2020; Kölker et al., 2022;

[4] We are aware of some limitations in relation to GenIDA data on seizures reported from caregivers. In the future the GenIDA questionnaire is planned to be modified with the ILAE classification of seizures (https://www.ilae.org/).

Hearing problems	Prevalence [%]	YES / NO	n=
Recurrent ears infections	20.4		60
Deafness (no devises)	9		60
Ear malformation	3.6		60
Other	12.6		60

Fig. 16. Hearing problems.

Treatments for hearing	YES / NO / I don't know	n=
Hearing aids		38
Hearing tubes (ear or tympanostomy tubes)		42

Fig. 17. Treatments for hearing.

Respiratory and pulmonary problems	Prevalence [%]	YES /NO / I don't know	n=
Asthma	5.1		17
Hay fever (Allergic rhinitis)	4.5		17
Respiratory rate problem	3.2		18
Pulmonary function problem	3.2		17
Tracheomalacia	2.6		16
Laryngomalacia	2.6		15

Fig. 18. Respiratory and pulmonary problems.

Renal, bladder and urogenital problem	Prevalence [%]	YES /NO / I don't know	n=
Renal malformation (e.g. horseshoe kidney)	5.2		19
Urinary system malformation	5.2		20
Frequent urinary tract infection	4.5		22
Undescended testicles (cryptorchidism)	4.5		20
Insufficient renal function	3.9		18
Vesico-ureteral reflux (VUR)	3.9		22

Fig. 19. Renal, bladder and urogenital problems.

Rüther et al., 2016), especially in relation to orphan drug development and clinical outcomes for rare diseases trials (Kolochavina, 2021; Whittal et al., 2021).

The ability to collect data from a geographically diverse and limited patient population is essential for understanding the multifaceted nature of KLEFS1. The international representation of the cohort, encompassing regions such as Europe, North and South America, Australia, New Zealand, Asia, and Africa, enhances the generalizability of the findings. However, there is a limitation of study due to high participation of mainly western countries, which introduces data bias and inequality. Although we are aware of this bias, we have no direct influence on it, as it represents a wider problem also linked to different health systems and socio-cultural causes of (non)participation.

Our study delves into various aspects of KLEFS1, showing clinical symptoms and their prevalence. All together 25 different groups of clinical symptoms are observed and 20 of them are frequently reported

Epilepsy	Prevalence [%]	YES /NO / I don't know	n=
Absence seizures	9		28
Grand mal seizures (Tonic-clonic seizures)	9		30
Febrile convulsions (fever fit)	6.4		28
Complex partial seizures (focal seizures)	6.4		25
Nocturnal seizures	5.1		26
Infantile spasms	3.8		25
Simple partial seizures (focal seizures)	3.8		26
Atonic (drop) seizures	2.6		23
Lennox-Gastaut syndrome	1.9		25
Unknown type of seizures	3.2		24

Fig. 20. Epilepsy - seizure types.

Endocrine and metabolic problems	Prevalence [%]	YES / NO	n=
Hypothyroidism (under-active thyroid)	7.1		21
Hyperthyroidism (over-active thyroid)	1.9		21
Growth hormone deficiency	0.6		21
Other	5.2		21

Fig. 21. Endocrine and metabolic problems.

Vascular problems	Prevalence [%]	YES / NO	n=
Hypertension	1.3		13
Hypotension	0.6		13
Coagulation problems	0.6		13
Other	6.5		13

Fig. 22. Vascular problems.

in KLEFS1 (from behaviour problems to epilepsy) with the prevalence range 67%–25%. The top 10 most frequently reported clinical symptoms are behaviour problems (67.1%), vision problems (66.9%), problems during newborn period (66.5%), walking problems (65.6%), diagnosis of ID (64.7%), problems during pregnancy/labour/delivery (62.6%), musculo-skeletal problems (57.3%), digestive problems (57.1%), feeding problems (52.1%) and sleeping disorders (50%).

Behavioural problems are frequently reported by caregivers, underlining the need for comprehensive management strategies. It is known that some KLEFS1 individuals are also experiencing sudden regression of functioning mostly during adolescence (Vermeulen, de Boer, et al., 2017; Vermeulen, Staal, et al., 2017), that is why we propose to add additional questions to the Genida questionnaire dedicated to the topic of regression for further research. Vermeulen, de Boer, et al. (2017) also state, that KLEFS1 patients who suffered from regression showed high symptom scores of psychosis, but this was not recognized in most of them and none of the patients received optimal antipsychotic treatment. With this in mind, the authors state that there may be a high prevalence of psychosis in KLEFS1. In this study most reported behaviour problems in KLEFS1 in our study are repetitive behaviour (54.4%), attention deficit (52.5%), anxiety (46.2%), impulsivity (44.9%), restricted interests (44.9%), self-aggressiveness (44.3%), obsessions (43%), aggressive behaviour (38.6%), hyperactivity (29.7%), phobias (27.2%), depressive tendencies (20.3%) and also shyness (18.4%). Diagnosed schizophrenia has a low prevalence of 1,3%, but as the mean age of KLEFS1 individuals in the study is low (12.92 years), we need to be cautious as the number for all behaviour problems could be higher in adult population.

In this study the ASD prevalence was 27.1%, however, high prevalence of nearly 100% ASD was found in KLEFS1 based on the validated

ADOS (clinical) assessment (Vermeulen, de Boer, et al., 2017). The much lower numbers could be, because many individuals are not officially tested and thus did not receive the diagnosis.

Following, sleeping disorders are frequent in KLEFS1 individuals, representing a prevalence of 50%. The level of seriousness was mostly evaluated as major (40.5%) and moderate (39.2%), followed by mild (20.3%). As regression after severe sleep disturbances were observed in KLEFS1 individuals previously (Vermeulen, Staal, et al., 2017), sleep problems are an important clinical symptom to observe carefully.

The study also reveals notable findings related to the newborn period, with a significant proportion experiencing feeding difficulties (43.9%), neonatal hypotonia (37.8%), and jaundice (29.3%). These insights into early-life challenges are crucial for understanding the natural history of KLEFS1 and informing early intervention strategies.

Similarly, vision problems, predominantly hypermetropia with a high prevalence of 39.2%, and walking difficulties, with unstable gait (prevalence of 31.8%) being a notable concern, emphasize the multisystem nature of KLEFS1. Our investigation into musculoskeletal, digestive and feeding provides a comprehensive view of the diverse clinical features associated with KLEFS1. Musculoskeletal problems (57.3%), including joint laxity (21%) and pes planus (19.7%), highlight the impact on physical health and medical rehabilitation interventions, while digestive issues (57.1%) such as constipation (29.4%) and regurgitation (15.9%) underscore the need for targeted clinical management. Especially constipation is of concern, as it was mostly reported as major (41.5%), followed by moderate (28.3%) and mild (24.5%).

Study results show similar prevalence numbers as the ones in previous studies conducted (Bouman et al., 2024; Morison et al., 2024; Willemsen et al., 2012) based on clinical data and on smaller cohorts, what indicates that the CROs are a highly reliable data source. For instance, our study additionally verified that the majority of individuals operate within the moderate-to-severe range of ID, with only a small number experiencing mild ID, as originally stated from (Kleefstra and de Leeuw, 1993/2023). Also, looking at the abnormality of the cardiovascular system (short cardiac problems), we can see the same prevalence number of 40% in our study and in clinical data from Radboudumc in Vasireddi et al. (2024).

Furthermore, the prevalence of seizures and information about medication usage and effectiveness, offers valuable information for clinicians managing individuals with KLEFS1 and epilepsy. The study also explores endocrine and metabolic problems, providing insights into hypothyroidism and age at first menstruation. For instance, Bouman et al. (2024) studied growth, body composition, and endocrine-metabolic characteristics in individuals with KLEFS1, and the endocrine-metabolic investigations showed high thyroid dysregulation (22%). In our study, endocrine and metabolic problems were shown with a prevalence of 14.3%.

Limitations of this study include the reliance on solely caregiver-reported data, which may introduce reporting bias, and the potential for underreporting or incomplete information. Another limitation is the lack of proper representation across age ranges. This can lead to skewed results that may not accurately reflect the health outcomes or needs of the entire population. Additionally, the study does not include genotype data, which would enable to study genotype-phenotype correlations and could offer further insights into the heterogeneity of KLEFS1. However, Morison et al. (2024) have carried out a genotype-phenotype study and concluded that genotype and age do not explain the phenotypic variability. It should be taken into consideration that the study was focused on speech, language and cognition only. Future research endeavours should aim to address these limitations, further explore the genetic underpinnings of KLEFS1, and consider longitudinal assessments to better understand the natural history of the disorder.

The dropout in data collection needs to be mentioned. As the questions posed first have a high response rate (N = 172), later questions are increasingly left unanswered, showing that in sum 46 main questions including many sub-questions are maybe too many, because the participants give up sooner and don't answer all the questions.

While much attention is rightly focused on the individual's well-being, it's essential to recognize that these conditions often ripple through the entire family unit, profoundly influencing their quality of life. Unfortunately, this vital aspect is frequently overlooked, while Shah et al. (2021) focused on it, naming it *Family reported outcomes* (FROs), meaning studying the impact that a patient's illness or disability can have on their family members. This shift in perspective aligns with the ethos of person-centered healthcare, where the holistic needs of the individual and their support network are prioritized. It becomes evident that existing rare disease data collection and registries should extend beyond merely tracking individual health outcomes, but measuring also the quality of family life to gain a more comprehensive understanding of the broader impact of rare conditions. By integrating FROs into healthcare assessments, we not only gain insights into the challenges faced by families but also pave the way for more tailored and effective interventions. Ultimately, this approach fosters a more inclusive and compassionate healthcare system—one that recognizes and addresses the interconnectedness of health outcomes within the family unit.

All in all, this study could help with the development of meaningful endpoints for KLEFS1. Research of this nature, when combined with clinician-driven assessments, can prove beneficial in guiding the formulation of possible forthcoming clinical trials targeting KLEFS1.

In essence, this study not only advances our knowledge of KLEFS1 but also underscores the significance of collaborative, patient-centered research approaches in the rare disease domain. The caregiver-reported outcomes presented herein provide a foundation for ongoing discussions within the medical and research communities, paving the way for continued advancements in understanding, managing, and ultimately improving the lives of individuals with KLEFS1.

5. Conclusion and recommendations

In conclusion, this caregiver-reported study on KLEFS1 provides a comprehensive analysis of the clinical spectrum and prevalence of symptoms associated with this rare neurodevelopmental disorder. The utilization of an international cohort within the GenIDA online data collection platform has allowed for a detailed examination of the diverse manifestations of KLEFS1 across different geographical regions. The findings of this study contribute significantly to the understanding of KLEFS1, shedding light on the prevalence and severity of various symptoms, ranging from intellectual disability and behavioural problems to musculoskeletal, digestive, and sensory issues. The inclusion of demographic information enhances the generalizability of the results, capturing the global heterogeneity of KLEFS1.

However, it is crucial to acknowledge the limitations inherent in the current data collection methods. The challenges encountered during data collection, the complexity of interpreting the outcomes, and the inability to leverage longitudinal data pose significant constraints. The potential benefits of a more sophisticated and technically robust database are evident, presenting opportunities for enhanced analysis and a deeper understanding of KLEFS1 over time.

As we appreciate the invaluable contributions of caregivers in providing essential data, it becomes apparent that optimizing the organization and structure of caregiver-reported registries is imperative. Recommendations for caregiver-reported registries include.

1. Database Enhancement: Consider technological improvements to enhance the database infrastructure, facilitating a more streamlined and efficient data collection process.
2. Longitudinal Data Integration: Explore solutions to incorporate and utilize longitudinal data effectively. This would allow for a more nuanced understanding of the progression of KLEFS1 symptoms over time.
3. User-Friendly Interface: Design a user-friendly interface for caregivers, ensuring a seamless and positive experience during data

input. Clear instructions and intuitive design elements can enhance the quality and completeness of the collected data.
4. Collaboration with AI Technologies: Explore collaborations with artificial intelligence technologies to supplement data collection efforts. Automated analysis could offer additional insights and complement traditional data collection methods.
5. Regular Updates and Communication: Establish a system for regular updates and communication with caregivers. Keeping caregivers informed about the impact of their contributions and the progress of the research fosters a sense of involvement and appreciation.

While acknowledging these recommendations, it is important to emphasize that caregivers play a pivotal role in advancing rare disease research. The suggestions provided aim to enhance the efficiency and impact of caregiver-reported registries, ultimately contributing to a more comprehensive and dynamic understanding of rare disorders like KLEFS1.

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CRediT authorship contribution statement

Tanja Zdolšek Draksler: Writing – review & editing, Writing – original draft, Resources, Methodology, Data curation, Visualization, Conceptualization. **Arianne Bouman:** Writing – original draft, Methodology. **Alenka Guček:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. **Erik Novak:** Data curation. **Pauline Burger:** Resources. **Florent Colin:** Resources. **Tjitske Kleefstra:** Writing – original draft.

Declaration of competing interest

Authors have no relevant conflicts of interest to disclose.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Benjamin, K., Vernon, M.K., Patrick, D.L., Perfetto, E., Nestler-Parr, S., Burke, L., 2017. Patient-reported outcome and observer-reported outcome assessment in rare disease clinical trials: an ISPOR COA emerging good practices task force report. Value Health 20 (7), 838–855. https://doi.org/10.1016/j.jval.2017.05.015.

Bloemendaal, M., Vlaming, P., De Boer, A., Vermeulen-Kalk, K., Bouman, A., Kleefstra, T., Arias Vasquez, A., 2023. The role of the gut microbiota in patients with Kleefstra syndrome. Am. J. Med. Genet. Part B: Neuropsychiatric Genetics 192 (7–8), 124–138. https://doi.org/10.1002/ajmg.b.32926.

Bose, M., Cuthbertson, D.D., Fraser, M.A., Roullet, J.-B., Gibson, K.M., Schules, D.R., Gawron, K.M., Gamble, M.B., Sacra, K.M., Lopez, M.J., Rizzo, W.B., 2020. Zellweger spectrum disorder: a cross-sectional study of symptom prevalence using input from family caregivers. Molecular Genetics and Metabolism Reports 25, 100694. https://doi.org/10.1016/j.ymgmr.2020.100694.

Bouman, A., Geelen, J.M., Kummeling, J., Schenck, A., Van Der Zwan, Y.G., Klein, W.M., Kleefstra, T., 2024. Growth, body composition, and endocrine-metabolic profiles of individuals with Kleefstra syndrome provide directions for clinical management and translational studies. Am. J. Med. Genet. 194 (5), e63472 https://doi.org/10.1002/ajmg.a.63472.

Burger, P., Colin, F., Strehle, A., Mazzucotelli, T., Collot, N., Coutelle, R., Durand, B., Bouman, A., Landau Prat, D., Kleefstra, T., Parrend, P., Piton, A., Koolen, D.A., Mandel, J.-L., 2023. GenIDA: an international participatory database to gain knowledge on health issues related to genetic forms of neurodevelopmental disorders. J. Neural. Transm. 130 (3), 459–471. https://doi.org/10.1007/s00702-022-02569-3.

Ciani, O., Federici, C.B., 2020. Value lies in the eye of the patients: the why, what, and how of patient-reported outcomes measures. Clin. Therapeut. 42 (1), 25–33. https://doi.org/10.1016/j.clinthera.2019.11.016.

Kleefstra, T., de Leeuw, N., 1993. Kleefstra syndrome. In: Adam, M.P., Feldman, J., Mirzaa, G.M., Pagon, R.A., Wallace, S.E., Bean, L.J., Gripp, K.W., Amemiya, A. (Eds.), GeneReviews®. University of Washington, Seattle. http://www.ncbi.nlm.nih.gov/books/NBK47079/.

Kölker, S., Gleich, F., Mütze, U., Opladen, T., 2022. Rare disease registries are key to evidence-based personalized medicine: highlighting the European experience. Front. Endocrinol. 13, 832063 https://doi.org/10.3389/fendo.2022.832063.

Kolochavina, M., 2021. The case for real-world data and real-world evidence generation in rare and orphan medicinal drug development. In: Huml, R.A. (Ed.), Rare Disease Drug Development. Springer International Publishing, pp. 371–387. https://doi.org/10.1007/978-3-030-78605-2_24.

Morison, L.D., Kennis, M.G.P., Rots, D., Bouman, A., Kummeling, J., Palmer, E., Vogel, A. P., Liegeois, F., Brignell, A., Srivastava, S., Frazier, Z., Milnes, D., Goel, H., Amor, D. J., Scheffer, I.E., Kleefstra, T., Morgan, A.T., 2024. Expanding the phenotype of Kleefstra syndrome: speech, language and cognition in 103 individuals. J. Med. Genet. https://doi.org/10.1136/jmg-2023-109702 jmg-2023-109702.

Rüther, A., Elstein, D., Wong-Rieger, D., Guyatt, G., 2016. Aspects of patient reported outcomes in rare diseases: a discussion paper. Int. J. Technol. Assess. Health Care 32 (3), 126–130. https://doi.org/10.1017/S0266462316000271.

Shah, R., Ali, F.M., Finlay, A.Y., Salek, M.S., 2021. Family reported outcomes, an unmet need in the management of a patient's disease: appraisal of the literature. Health Qual. Life Outcome 19 (1), 194. https://doi.org/10.1186/s12955-021-01819-4.

Vasireddi, S.K., Draksler, T.Z., Bouman, A., Kummeling, J., Wheeler, M., Reuter, C., Srivastava, S., Harris, J., Fisher, P.G., Narayan, S.M., Wang, P.J., Badhwar, N., Kleefstra, T., Perez, M.V., 2024. Arrhythmias including atrial fibrillation and congenital heart disease in Kleefstra syndrome: a possible epigenetic link. Europace 26 (1), euae003. https://doi.org/10.1093/europace/euae003.

Vermeulen, K., de Boer, A., Janzing, J.G.E., Koolen, D.A., Ockeloen, C.W., Willemsen, M. H., Verhoef, F.M., van Deurzen, P.A.M., van Dongen, L., van Bokhoven, H., Egger, J. I.M., Staal, W.G., Kleefstra, T., 2017a. Adaptive and maladaptive functioning in Kleefstra syndrome compared to other rare genetic disorders with intellectual disabilities. Am. J. Med. Genet. 173 (7), 1821–1830. https://doi.org/10.1002/ajmg.a.38280.

Vermeulen, K., Staal, W.G., Janzing, J.G., van Bokhoven, H., Egger, J.I.M., Kleefstra, T., 2017b. Sleep disturbance as a precursor of severe regression in Kleefstra syndrome suggests a need for firm and rapid pharmacological treatment. Clin. Neuropharmacol. 40 (4), 185. https://doi.org/10.1097/WNF.0000000000000226.

Whittal, A., Meregaglia, M., Nicod, E., 2021. The use of patient-reported outcome measures in rare diseases and implications for health technology assessment. The Patient 14 (5), 485–503. https://doi.org/10.1007/s40271-020-00493-w.

Willemsen, M.H., Vulto-van Silfhout, A.T., Nillesen, W.M., Wissink-Lindhout, W.M., van Bokhoven, H., Philip, N., Berry-Kravis, E.M., Kini, U., van Ravenswaaij-Arts, C.M.A., Delle Chiaie, B., Innes, A.M.M., Houge, G., Kosonen, T., Cremer, K., Fannemel, M., Stray-Pedersen, A., Reardon, W., Ignatius, J., Lachlan, K., et al., 2012. Update on Kleefstra syndrome. Mol. Syndromol. 2 (3–5), 202–212. https://doi.org/10.1159/000335648.