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International Clinical Evidence-based Guideline for Kleefstra Syndrome

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International Clinical Evidence-based Guideline for Kleefstra Syndrome

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Abbreviations

- AAC: Augmentative and alternative communication
- ASD: Autism spectrum disorder
- CMA: chromosome micro array
- CNVs: copy number variants
- CVI: Cerebral Visual Impairment
- DSM-5: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
- ECG: Electrocardiogram
- EEG: Electroencephalography
- ES: exome sequencing
- FISH: Fluorescence in situ hybridization
- GS: genome sequencing
- ID: intellectual disability
- KLEFS1: Kleefstra syndrome type 1
- mNDD: Monogenic neurodevelopmental disorder
- PAS-ADD: Psychiatric Assessment Schedule for Adults with Developmental Disabilities
- SNVs: single nucleotide variants

Abstract

Kleefstra syndrome (KLEFS1) is a rare monogenic neurodevelopmental disorder (mNDD) with multisystem involvement, caused by disruption of EHMT1 function, resulting in significant burden on affected individuals and their families. The current shortage of and globally scattered syndrome-specific knowledge has led to significant disparities in the access to and provision of evidence-based and individual-centered expert care.

To address the challenges and improve outcomes for individuals with KLEFS1, an international KLEFS1 guideline consortium was formed consisting of 43 participants, both clinical experts and

patient-representatives, from 15 different countries. The primary goal of the consortium was to develop a comprehensive and high-quality guideline for KLEFS1, aiming to enhance patient care, establish a uniform minimum international standard of care, and support decision-making.

The current clinical guideline is evidence-based and includes 66 tailored recommendations to improve KLEFS1 care. The comprehensive methodological approach ensures broad consensus and supports effective implementation. Furthermore, this guideline serves as a valuable methodological model for guideline development in the context of rare disorders.

Keywords International Clinical Guideline, KLEFS1, recommendations,

Introduction

Kleefstra syndrome (KLEFS1) (OMIM #610253) is a rare monogenic neurodevelopmental disorder (mNDD) with an estimated prevalence of ~1:36,000 [1]. The recognizable syndrome was initially identified by detecting subtelomeric deletions affecting 9q34 in affected individuals and was consequently termed ''9q-subtelomeric-deletion syndrome'' in the late 1990s. In 2006, Kleefstra et al. identified loss-of-function of *EHMT1* (HGNC:24650) located at the 9q34.3 chromosomal locus as responsible for the features of this syndrome [2]. KLEFS1 is mainly characterized by intellectual disability in the mild to profound spectrum, global developmental delay, autism spectrum disorder, and distinctive facial features. Additionally, the syndrome is frequently associated with abnormalities affecting multiple organ systems and a variety of mental health and behavioral issues [1, 3].

Despite the increased understanding of both the genotypic and phenotypic spectra of KLEFS1, knowledge remains fragmented and variably implemented. Consequently, gained knowledge often fails to improve clinical care, screening, surveillance, and follow-up. KLEFS1 is one of many rare genetic mNDDs for which clinicians often face significant challenges providing optimal medical management, with a clear contributor being the scarcity of comprehensive and high-quality clinical guidelines [4].

Therefore, a consortium consisting of international experts and patient-representatives aimed to implement impactful, evidence-based clinical recommendations for KLEFS1, in order to enhance clinical care for individuals with KLEFS1, establish a uniform minimum standard of care, and to support shared decision making about investigations and evidence-based therapies in general. Moreover, this evidence-based guideline may serve as an important methodological example of guideline development for rare mNDDs. The naming by OMIM of Kleefstra syndrome type 2 (OMIM #617768), caused by pathogenic variants in *KMT2C* (HGNC:13726) , had gained much confusion. The

scope of the current activities are solely directed to KLEFS1 (EHMT1) as it has been convincingly shown that both are distinct conditions [5].

The main outcome of the current guideline approach are 66 clinical recommendations for KLEFS1 that are based on available current evidence, standalone, unambiguous, and universally applicable across countries. The target audience of this guideline are clinicians and paramedical professionals treating KLEFS1 individuals worldwide, and the recommendations may be of support for the community in a broader sense. Recommendations were critically appraised during a face-to-face meeting, involving professionals and patient-representatives. This comprehensive approach ensures broad consensus, that further facilitates effective implementation.

Methods

The established consortium consisted of 34 clinical experts ((Clinical) geneticists, pediatricians, psychiatrists, psychologists, neurologists, neuroscientists, a cardiologist, genetic counsellors, speech-language therapists, (clinical) researchers, and a guideline methodologist), and 10 parents of an individual with KLEFS1, from 15 different countries (Australia, Canada, China, Denmark, France, Germany, Hungary, Italy, Latvia, the Netherlands, Poland, Slovenia, Spain, United Kingdom, and United States). One of the professionals was also a parent. Consortium members were recruited through different networks aiming to achieve diversity in specialties, countries and perspectives. The guideline development was initiated at Radboud University Medical Center, Nijmegen, the Netherlands, a center of expertise where many KLEFS1 individuals receive care. The consortium was divided into five working groups based on the main clinical topics. Each working group had at least 2 clinical specialists and one patient representative involved. A coordinating group was formed to coordinate the guideline development process and monitor quality and uniformity between the working groups. This group consisted of the chair and clinical geneticist (TK), a clinical genetics researcher (AB), a patient representative (with professional guideline experience as a medical librarian at the Dutch College of General practitioners) (CSE), a project manager (KV), and a guideline methodologist (CG).

The AGREE II framework was followed in the development of the guideline. Additionally, the GRADE approach was applied where possible. The scarcity of evidence forced a couple of adjustments, mainly for grading the evidence (see discussion paragraph).

The priority-setting for topics was performed with the application of the recently developed re-weighted priority-setting (REPS)-tool [6]. First, 45 clinical topics were identified through discussion in working groups and then reviewed by the entire consortium. Thirty-one panel members (including 10

patient representatives) assigned priority scores on each of the topics through a survey, based on the following criteria: unwanted (international) clinical variance that may be improved with this guideline; high prevalence of symptoms; high (disease) burden (either for the KLEFS1 population or for their families. With families, we mean family members, caregivers, and/or other directly involved individuals). Based on this, 12 areas in need of clinical management recommendations were prioritized.

Systematic literature searches were performed in four relevant bibliographic databases (PubMed, Embase, CINAHL and PsycINFO) without language restrictions in November 2022 and updated in May 2024 to identify articles about KLEFS1. All search strategies were designed by a medical librarian with experience in literature searches for medical guidelines and included a combination of database-adapted subject headings and title/abstract search-terms, including Kleefstra syndrome, EHMT1, 9q34, and 9qSTDS. Details about the search strategies are presented in Appendix I; See Figure 1 for the PRISMA diagram of the search strategy results. Backward citation screening was used as an additional search strategy and consortium members were able to submit relevant articles, to minimize the risk of missing articles. We used only one inclusion criterion due to the expected limited amount of literature: any study involving clinical data on KLEFS1 was included.

Two authors selected the relevant articles based on title and abstract (AB and CSE), and the full-text selection was performed by the working group members dedicated to their specific topic. Exclusion criteria were: (1) fundamental studies/*in vitro* studies/animal models, (2) narrative reviews without clinical data, (3) no clinical data reported on one of the prioritized clinical questions/guideline topics.

The relevant articles were selected and summarized for each clinical management topic. The quality of the evidence was evaluated per included study. GRADE analyses were not applied as the quality of the evidence was very low for every topic, due to the limited data and the nature of the research designs (most were case studies).

To further incorporate the input from the families of individuals with KLEFS1, two separate online focus groups of an hour each were organized. The focus groups took place in January 2024. In the first focus group, four patient representatives participated, and the discussed topics were sleep, medication use and regression. Five patient representatives participated in the second focus group about diagnostic evaluation, support for families, and constipation. Two patient representatives participated in both focus groups. A summary of discussed themes and important take-aways was presented at the consensus meeting (provided in Supplementary file: focus groups parents).

The consensus meeting took part on February 15, 16 and 17, 2024 in the Erasmus Medical Center, Rotterdam, the Netherlands. Of the consortium, 33 members were present, including 6 patient representatives. Of these 33, 10 members of the consortium participated online. All participants signed a conflict of interest statement. During the meeting, literature and expert input were presented by the experts from the working groups, reviewed, and recommendations were drafted and discussed until consensus was reached. Following the consensus meeting, a final online voting round on the recommendations was conducted, in which participants of the consensus meeting could take part. For all recommendations, 27 consortium members (of which 4 were patient representatives) voted to either agree, disagree or abstain. Complete agreement (100%) was rated level A agreement, for high agreement at least 95% of the members needed to vote agree, good agreement was reached with 90% of the votes. The multidisciplinary discussions and voting ensured that different perspectives were weighed in the rationale; evidence, expertise, lived experiences and feasibility of each recommendation itself and the set of recommendations as a whole.

[Insert Figure 1 here]

Results

Genetic testing

Kleefstra syndrome (KLEFS1) is caused by pathogenic loss-of-function variants affecting the *EHMT1* gene: intragenic or due to a 9q34.3 deletion affecting at least part of *EHMT1* [7]. Approximately 50% of the individuals have pathogenic single nucleotide variants (SNVs) or indels [1, 7] and ~50% have copy number variants (CNVs) [1, 7] while a small number of individuals have other structural variants (e.g., intragenic duplications, translocations, or other complex structural changes affecting *EHMT1* [1, 8-10]). Therefore, application of different diagnostic methods may be necessary to detect all variant types.

The vast majority (~80-90%) of the pathogenic SNVs/indels so far reported are truncating variants (nonsense, frameshift and splice variants) and only 10-20% of variants are missense or in-frame indels, potentially altering the protein's function. Truncating variants are reported throughout the gene [1, 7, 11]. However, truncating variants in the first two exons may escape nonsense-mediated decay and there is a known alternative start codon in the third exon [1, 12]. Pathogenic protein altering variants have only been reported to date in the two functional ANK-repeat and (pre-/post) SET domains [1, 7, 11, 13]. The rest of the protein is largely disordered (regions lacking three-dimensional structure) and, therefore, the pathogenicity of *EHMT1* truncating variants in the first two exons and protein altering variants outside the ANK-repeat and SET domains should be interpreted with caution [1, 12]. Likewise important, it has been shown that *EHMT1* whole gene duplications (via triplosensitivity) are not associated with KLEFS1, but with a different non-syndromic neurodevelopmental disorder [14, 15].

As the majority of the KLEFS1 causative variants are intragenic, we endorse the application of (clinical) genome sequencing (GS) or (clinical) exome sequencing (ES) as the first-line test for individuals with neurodevelopmental disorders as suggested by expert consensus and ACMG guidelines [16, 17]. Trio analysis is preferred with the inclusion of CNVs and other structural variant detection. Alternatively, the test strategy can also be chromosomal micro array (CMA) followed by (trio) ES/GS (as indicated in Figure 2) depending on the local situation. Trio analyses have higher diagnostic yield, confirm parentage, accelerate the diagnostic process, and enhance certainty in variant classification, particularly by identifying *de novo* variants [18-20].

When application of GS/ES fails to yield a diagnosis but clinical suspicion for a KLEFS1 diagnosis remains high, other approaches should be considered when available, such as *EHMT1* gene-targeted multiplex ligation-dependent probe amplification (MLPA) or exon-level (e.g., XON) array analyses. CNVs, including deletions and duplications, might be entirely intragenic and approximately 5% are

single-exon CNVs [1] and might be overlooked by a genome-wide testing methods due to the small size.

[Insert Figure 2 here]

KLEFS1 is known to be associated with a specific DNA methylation signature (episignature) [21, 22]. This test is available in standardized clinical settings (e.g., EpiSign test) [21] or in research settings (e.g., by using open-access portal EpigenCentral) [23]. DNA methylation signature's testing results should only be interpreted together with the individual's phenotype and genotype [24-26]. Additionally, variant effect predictions on protein three-dimensional structure can provide insights into their potential impact [1, 27].

Other research-oriented tests include EHMT1 functional assessment of missense variants for their impact on protein enzymatic activity [1, 13, 28]. Furthermore, in patient-derived induced neurons, neuronal network patterns associated with KLEFS1 can be evaluated using microelectrode array in the research setting [29].

KLEFS1 has full penetrance and is inherited in an autosomal dominant manner. While most pathogenic variants arise *de novo,* documented cases include inheritance from parents with gonadosomatic or gonadal mosaicism [30-33], balanced chromosomal aberrations [10], and familial pathogenic KLEFS1 cases with a milder phenotype [1].

Both parents of an individual with an unbalanced translocation affecting 9q34.3 should be offered a test for balanced translocation: karyotype and/or FISH (depending on size) or, if available, long-read sequencing. For large (>50-100kb) interstitial CNVs, it is recommended to analyze both parents using a FISH probe targeting *EHMT1* in addition to G-band karyotyping or use long-read sequencing [34].

KLEFS1 can be diagnosed prenatally if genetic testing is organized due to congenital anomalies identified on ultrasound, increased nuchal translucency, or possibly altered maternal serum markers [35-37]. However, the full prenatal phenotypic spectrum and the frequencies of anomalies remain unknown, as systematic studies are lacking. Prenatal testing typically involves CMA and/or ES/GS, but approaches may differ depending on available methods, local or institutional guidelines, and laws.

KLEFS1 has characteristic, but not always specific, clinical manifestations. Clinical overlap with other neurodevelopmental disorders can be observed, such as those caused by pathogenic variants in *KMT2C*, *MBD5* or *SMARCB1*, that are part of the chromatinopathy subclass of mNDDs.

Genotype-phenotype correlations have been identified for several symptoms [1, 3, 38]: Individuals with (large) multigene variants exhibit more severe intellectual disability (ID) and a higher prevalence

of constipation and short stature. Those with a protein altering variant in the ANKR or SET domain typically have higher IQs, fewer feeding difficulties, less structural heart defects, and less recurrent infections in comparison to truncating variants. Variants in the N-terminal domain are associated with an overall milder phenotype, including lower prevalence of ID, global developmental delay, constipation, and feeding difficulties.

Counselling

Upon identification of an *EHMT1* variant, it is recommended to refer the individual to a clinical genetics team or, alternatively, to a clinician specialized in genetic NDDs. This consultation is crucial for phenotype evaluation, diagnosis confirmation, and genetic counselling. Counselling should cover education about the condition and the genetic cause, the inheritance pattern, recurrence risk, and reproductive options [39, 40]. Information on available therapeutic options, the associated phenotypic spectrum, genotype-phenotype correlations and variability should also be provided. Additionally, guidance on appropriate health checks and the natural history of the condition should be offered.

Information provided should be tailored to the preferences of the individual/family, addressing their main concerns and coping abilities. Follow up appointments to address questions that individuals/families have across the lifespan should be provided. Lastly, details about patient/advocacy organizations, peer-support groups, counselling services, and reliable sources of information should be proactively offered (see supplement: List of clinical and patient expertise centers).

As KLEFS1 is a rare mNDD with high burden on patients and families, clinicians should regularly inquire about the emotional well-being of individuals and their families and offer suitable support and resources. Additionally, clinicians should proactively discuss and plan the transition to adult care, starting in mid-adolescence. During these longitudinal visits, clinicians are recommended to continue d to seek expert advice on KLEFS1 to enhance management, data collection efforts, and connect to the latest updates on evidence-based care, clinical trials, and new therapies.

Mental health-Behavior

Mental health disorders and behaviors in KLEFS1 are highly prevalent, with a cumulative lifetime prevalence of 74% (237/320, 95%CI 69-79%) [1, 7, 41-43] (Supplementary Table 1). Behavioral issues typically manifest in childhood, while mental health disorders primarily emerge in adolescence and adulthood. Autism spectrum disorder (ASD) affects the majority of the individuals with KLEFS1 [1, 44]. The most common mental health disorders include anxiety, depressive disorder, and obsessive-

compulsive and related disorders. Additionally, regression occurs in a significant proportion of individuals with KLEFS1 *(see chapter Regression).*

Mental health disorders and behaviors often coexist, leading to a substantial burden on individuals, parents, and support systems (Unpublished data, ErasmusMC) [45, 46]. This burden affects daily living, increases parental stress, and may require institutional care [45, 46].

Regular monitoring and follow-up of mental health and behavioral status are essential due to their high prevalence and the frequent changes in symptoms and specific disorders over time [1, 3, 44, 47]. Therefore, we recommend annual screening in primary or pediatric clinics for early detection of changes in behavior or mental health symptoms, medication use, co-occurring conditions (e.g., constipation, regression), and current management and support provisions. It is crucial to consider somatic comorbidities (e.g., hypothyroidism, vitamin deficiencies) as potential contributors to psychiatric symptoms [48].

Referral to psychiatric or psychological care is advised when functional impairment (e.g., in personal care, social relationships, work/school performance, daily activities) or suspected conditions outlined in the DSM-5 are present. Mental health professionals should conduct diagnostics, provide education, personalized intervention or treatment, and ensure follow-up.

The following instruments are recommended in KLEFS1 for the diagnosis and monitoring of intellectual disability, developmental delay, and mental health symptoms [31, 44, 45, 49]: Vineland Adaptive Behavior Scales, Mini PAS-ADD, Autism Diagnostic Observation Schedule, Child Behavior Checklist, Cambridge Neuropsychological Tests Automated Battery, Wechsler Intelligence Scale for Children, or other intelligence quotient test.

Regression

Episodes of sudden decline in functioning during adolescence and adulthood are frequently reported in KLEFS1 (range 11-50%) [1, 3, 38, 44, 46] (Supplementary Table 1), often referred to as (developmental) regression. Based on current insights we suggest to define this as ‘’An absolute decline of functioning in at least one of the adaptive behavior domains of practical, conceptual or social-emotional skills in the individual, which -if left untreated - would last at least several months.” [50].

The regressive episodes may be preceded by severe sleep problems or stressors, which may have both physical (e.g., pneumonia or hormonal changes) and psychological (e.g., moving house, loss of a family member) origins [3, 51] (unpublished data, Radboudumc). Studies indicate decline in speech-

language, motor, social-emotional, and cognitive skills during regression, frequently accompanied by aggressive outbursts, temper tantrums, and sleep problems [3].

In cases of regression, referral to psychiatry is recommended. Additionally, neurological and pediatric evaluation should be included in the etiological assessment and screening for treatable medical conditions *(see chapter Neurology and chapter Growth-metabolism)*. Psychiatric evaluation is crucial for diagnostics, treatment, and follow-up, ensuring differentiation from conditions like psychosis and catatonia. Psychotropic drugs, particularly olanzapine, have shown effectivity in some reported individuals [44, 52] (unpublished data, Radboudumc). Aripiprazole, and the combination of risperidone and haloperidol, has been shown effective in some cases, but caution is warranted due to potential adverse effects such as tardive dyskinesia and weight gain. Zuclopenthixol, pipamperone, and benzodiazepines were found ineffective. Benzodiazepines have caused paradoxical agitation [43, 44, 52]. However, in cases with catatonia, benzodiazepines are first choice according to regular guidelines, and for the KLEFS1 population. Regular assessment of adaptive functioning using the Vineland Adaptive Behavior Scales is crucial to effectively track changes during and after a regressive episode [44].

Neurology

In addition to mental health and behavioral issues, KLEFS1 is frequently associated with neurological abnormalities, epilepsy and sleep disturbance. Structural brain abnormalities are observed in approximately half of individuals [1], but they are most often non-specific and do not require specific intervention. They encompass, but are not limited, to the following: white matter abnormalities, ventriculomegaly, cerebral cysts, and cerebral, cerebellar, brainstem, and corpus callosum atrophy or hypoplasia [1, 53, 54]. In some cases, T2 hyperintensities of the white matter may be reversible with expectant management [1, 55].

Routine investigation for structural brain abnormalities in KLEFS1 is not clinically indicated. However, a brain MRI should be considered when there are focal seizures, focal findings on electroencephalography (EEG), developmental regression, or acute onset of psychosis or catatonia. The use of gadolinium with MRI should be considered based on clinical indications. Follow-up MRI may be warranted for actionable findings identified on the initial MRI or when clinical changes occur.

Epilepsy occurs in 10-44% of individuals with KLEFS1 [1, 3, 7, 38, 41, 43, 46, 56] (Supplementary Table 1) with no significant differences based on genotype, severity of developmental delay, or gender [1, 54]. Individuals should be referred to a neurologist or epilepsy specialist if they have suspected or confirmed epilepsy. A routine EEG is recommended for initial assessment if there are concerns for seizures or epilepsy, or if there is developmental regression. Video recording that captures both

awake and asleep periods should be included given the possibility of (developmental) epileptic encephalopathy with spike-and-wave activation in sleep, especially if there is language or cognitive regression [57].

Onset of seizures in KLEFS1 commonly starts in childhood and can occur in infancy [2, 58], childhood [2, 41], and adolescence [38, 43, 54]. Seizure types and frequency vary widely, with focal motor and tonic-clonic seizures being most common [1, 2, 41, 54]. Severe epilepsy syndromes such as infantile spasms and Lennox-Gastaut syndrome have been reported in some cases [38, 54, 59-61].

Earlier seizure onset (< 36 months) is associated with more frequent seizures [54]. Treatment should follow local protocols; valproic acid and carbamazepine have shown effectiveness [54]. Levetiracetam is commonly used as a first-line medication in child neurology practices, but it may induce behavioral irritability, requiring careful consideration in individuals with significant behavioral dysregulation. Adrenocorticotropic hormone and prednisolone are used for infantile spasms in KLEFS1, with some individuals not responding to drugs [54]. Longitudinal studies report reduction in prescribed medications and overall seizure frequency over time [54]. Discontinuation of antiseizure medications should be managed according to local protocols.

Sleep

Sleep-wake disorders are prevalent in KLEFS1 (55%, n=265/480, 95%CI 51-60%) [1, 3, 44, 46] (Supplementary Table 1), and result in significant burden for both individuals and families in their health and daily life functioning. Insomnia during sleep onset and maintenance insomnia are most frequently observed [3, 7, 31, 47, 62, 63]. Sleep apnea is reported in a minority of individuals with KLEFS1 [1], and motor restlessness during sleep can also occur [1, 64, 65].

Sleep disturbances in KLEFS1 may occur independently or in association with psychological and/or behavioral issues. Studies indicate that successful management of psychopathology may improve sleep [52, 66, 67]. Furthermore, suddenly worsening, severe insomnia may precede a regressive episode and could serve as an early warning [52, 63, 66, 67].

Similar to ASD, the etiology of sleep problems in KLEFS1 often correlate with behavioral factors such as anxiety, sensory processing issues, and abnormalities in the circadian sleep-wake cycle, potentially linked to reduced melatonin levels [68].

Given the common occurrence and impact of sleep-wake disorders in KLEFS1, guidance on sleep hygiene practices and individualized adjustments to daily and bedtime routines based on sensory profiles are essential. Referral to a sleep specialist is advised when sleep disturbances are reported. Diagnostic evaluations should encompass identifying the type of sleep disturbance and aim to

understand the underlying causes, including somatic, psychiatric, social, and environmental factors such as constipation, gastroesophageal reflux disease, sleep apnea, depressive disorder, psychotic disorder, post-traumatic stress disorder, nightmares, daytime physical activities, and bedtime routines [personal observation].

Treatment should be tailored to the specific sleep disorder and underlying cause. Pharmacological interventions, including melatonin, should only be prescribed and monitored by healthcare professionals. While evidence on optimal treatments for sleep-wake disorders in KLEFS1 is limited, melatonin has shown efficacy in delayed sleep phase syndrome [31]. Slow-release melatonin alone or in combination with Quetiapine or Trazodone has been effective for insomnia [64]. However, while slow-release melatonin has improved sleep in some cases, caution is advised due to reported paradoxical effects [64]. For sleep apnea, positional therapy or positive airway pressure therapy are recommended. Benzodiazepines have shown inconsistent efficacy and may have paradoxical effects [44, 62, 64].

Vision and hearing

Impairments in vision and hearing are common in individuals with KLEFS1. These issues can be caused by several underlying conditions and manifest with considerable variability. Since sensory input is crucial for development, early recognition and targeted interventions are essential to optimize developmental outcomes.

Hearing problems affect 37% (207/554, 95%CI 33-41%) of individuals with KLEFS1 [1, 3, 7, 38, 43, 46, 56] (Supplementary Table 1). Reported types of hearing loss include conductive, sensorineural, or mixed [3, 9]. Bilateral hearing impairment is most common [1], with asymmetrical hearing loss also frequently observed [3]. The severity of hearing impairment varies, predominantly being mild degree [3, 9, 65, 69]. Hearing loss has been detected in KLEFS1 from neonatal stages to adulthood, and some cases show progressive deterioration, unrelated to regressive episodes [3].

The high prevalence of conductive hearing loss might be partly attributed to recurrent ear infections 24% (94/395, 95%CI 20%-28%) [1, 3, 36, 43] (Supplementary Table 1). Additionally, cerumen impaction increases the risk of conductive hearing loss. A narrow external ear canal is likely prevalent in KLEFS1, which may also increase the risk of cerumen impaction. Furthermore, central auditory processing disorders are likely present, but under-reported. Furthermore, we are aware of two individuals with cholesteatoma (unpublished data, Radboudumc).

Ophthalmological disorders are prevalent in KLEFS1, primarily, refractory abnormalities such as (high) hypermetropia and strabismus. Some individuals with KLEFS1 have been diagnosed or suspected to

have cerebral visual impairment (CVI) [1, 38]; this may be caused by cerebral damage due to complications during cardiac surgery or as a result of severe epileptic insults [70].

Refractive abnormalities typically manifest early in life, while strabismus can be diagnosed at any age. Conditions like glaucoma may present in adulthood but seem not more common in KLEFS1 compared to the general population. Regular screening and monitoring of auditory and visual function are crucial for individuals with KLEFS1, tailored to their developmental stage, behavioral challenges, and sensory sensitivities.

During screenings, special emphasis should be placed on assessing potential central auditory processing disorders, refractive errors, strabismus, and impairment of visual functions related to CVI in case of cerebral damage. Auditory function and visual acuity assessments should follow local protocols, with heightened monitoring for auditory function and strabismus until age 6.

Speech-Language-Communication

KLEFS1 is associated with speech and language disorders, with several individuals acquiring limited verbal speech [71]. Speech, language, and communication abilities in KLEFS1 vary widely, with approximately 65% of individuals aged 3 and older being able to form sentences [3]. First words typically emerge after 2 years of age [3, 43, 52, 72-75]. Larger chromosomal deletions (>1Mb) are associated with more impaired speech and language skills compared to smaller (<1Mb) and intragenic variants [3, 41, 71, 76].

Nearly all verbal individuals with KLEFS1 (98%) have one or more speech disorder(s) [3, 77]. Common speech disorders include dysarthria [3, 47] and childhood apraxia of speech [3, 77, 78]. Additionally, articulation disorder, phonological delay, phonological disorder, and stuttering are observed. Speech is discordant with cognition and language skills [3].

Augmentative and alternative communication (AAC) is a broad term for methods of communication that are used to support or replace speech. Importantly, AAC does not impede speech development and should be strongly considered in KLEFS1. Aided ACC, high-tech aided ACC, and sign language has been shown to enhance, communication at young ages, and in older individuals whose speech abilities have been reduced due to a regressive episode.

All individuals with KLEFS1 should receive speech and language assessment by a speech therapist, covering all areas of language including receptive and expressive abilities. Assessments should be conducted at time of diagnosis and occur annually until age 12. In adolescence and adulthood, additional assessments should be performed when indicated, such as with new concerns or signs of regression.

Appropriate intervention for speech and language disorders is important and should be tailored to the specific speech-language diagnosis. Moreover, interventions should be tailored to individuals' specific cognitive, vision, hearing, and co-occurring neurodevelopmental conditions (e.g., ASD and sensory processing disorder). Interventions used in autistic individuals and other genetic conditions may also be beneficial for individuals with KLEFS1 [79-82].

Cardiology

KLEFS1 is associated with a wide range of congenital and acquired heart conditions, including septal and valve defects, and rhythm disturbances [1, 3, 38, 83]. Rhythm disturbances are particularly diagnosed in late adolescence [83] and warrant specific treatment.

Given the common occurrence of heart defects, all individuals with KLEFS1 should receive cardiac evaluation at genetic diagnosis using cardiac echocardiography. If cardiac MRI is available, it may be considered as well. Moreover, starting from age 18, annual screening with an electrocardiogram (ECG) is recommended to detect rhythm abnormalities.

It is recommended to consult a cardiologist when initiating new medications with potential cardiac implications (e.g., psychiatric, prokinetic, and certain neurological medications) to prevent induced rhythm disturbance. Urgently refer to a cardiologist if an ECG conducted in primary care shows abnormalities or if cardiorespiratory symptoms worsen, where further workup with cardiac imaging and/or ambulatory rhythm monitoring might be warranted. If there is an unexplained decline in neurocognition, mood, or functional status, cardiology problems also need to be considered in the differential diagnosis.

Manage structural heart defects and/or rhythm abnormalities as per local protocol. Based on case reports and database entries the majority of congenital heart defects in KLEFS1 were not clinically significant and required no major interventions. However, severe cardiac malformations which required surgery were observed. Individuals with rhythm abnormalities responded well to treatments recommended for the general population [83]. To reduce cardiovascular risk in KLEFS1, regular monitoring of weight and metabolism is advised [48] *(see chapter Growth-Metabolism)*.

Growth-Metabolism

KLEFS1 is associated with short stature, overweight/obesity, and microcephaly. Short stature is found in a minority of individuals [7, 48], usually with no specific underlying pathology identified [48]. In contrast, overweight and obesity is observed in the majority of individuals with KLEFS1 [48] and might be related to *EHMT1* haploinsufficiency as preclinical models have shown disrupted fat metabolism [84, 85] and metabolic dysregulation in response to stressors [86]. Risk factors for overweight might be present in KLEFS1, including apathy [66], overeating, psychotropic medication

side effects, and hypothyroidism [48]. Head circumference in KLEFS1 is typically reduced across all ages, with microcephaly observed in about half of adults [48].

Regular monitoring of height and weight is recommended for children and adolescents, using appropriate growth charts and comparison to target-height norms. Head circumference should be assessed at least until age 3. In adulthood, regular evaluations of weight, waist circumference, and BMI are important to detect obesity-related diseases and cardiovascular complications, allowing for early interventions and preventive efforts to be initiated.

For individuals exhibiting short stature, overweight/obesity, or microcephaly, diagnostic investigations should be conducted following local protocols. Advice regarding the importance of regular physical activity and healthy eating habits might be particularly relevant in KLEFS1. Involvement of a dietitian/nutritionist can be beneficial.

Additionally, specific recommendations for KLEFS1 include the evaluation of thyroid function, glucose metabolism, lipid spectrum, and vitamin status (vitamin D, B12, and folic acid) at the onset of puberty or when there are related symptoms. For children with obesity, these biochemical measurements should be conducted every 1-3 years starting from age 8. Evaluation of bone mineralization through hand radiographs or Dual Energy X-ray Absorptiometry (DEXA) scans should be considered at the onset of puberty and for individuals with multiple or unexpected fractures, with follow-up as per local protocols.

Constipation

Constipation affects approximately half of individuals with KLEFS1 [1, 3, 38, 46], and it can manifest at all ages, sometimes presenting in atypical ways (personal observation, focus group}. The severity ranges from mild to severe, occasionally requiring surgical intervention (unpublished data, Genida registry). Constipation results in high burden on individuals, and may severely impact mental health, sleep, and increase the risk of urinary tract infections (personal observation, focus group).

Since constipation is a multifactorial condition [87], it is important to identify potential etiological factors and tailor interventions accordingly. Individuals with KLEFS1 likely have genetic predisposition and reduced gastrointestinal motility (personal observation). Common contributing factors in KLEFS1 include hypotonia, delayed toilet training, low fluid intake, dietary restrictions, medications causing constipation, reduced physical activity, and hypothyroidism [48].

Early prevention and tailored interventions are important to reduce associated health complications. Local protocols should guide diagnostics and treatment of functional constipation. Typically, volume-increasing laxatives such as macrogol or psyllium fibers are used in KLEFS1, comparable to general

practice (unpublished data, Radboudumc database; personal observation). Consultation with a gastroenterologist is advised if constipation persists despite standard therapies.

[Insert Table 1 here]

Discussion

In this paper, the results of an international and interdisciplinary endeavor to establish the most important recommendations for KLEFS1 are presented, in total 66 (Table 1). The process extended over more than two years, reflecting the commitment to involve a wide array of clinical specialists and representatives of individuals with KLEFS1 worldwide in the process. Avoiding conflicts of interest is difficult in rare conditions, because involved clinical experts are also active in research or policy efforts [4]. We addressed this by transparent communication about the conflicts of interests, consulting experts from outside the field of KLEFS1 where necessary and through a combination of open multidisciplinary discussions in consensus formation with individual voting on recommendations.

We adhered to the AGREE II and GRADE methodology frameworks as best as possible, prioritized clinical topics, systematically searched for and selected literature, and complemented this by prevalence data, and expertise of international clinical and patient experts. The small number of published clinical studies allowed us to perform only one overarching literature search on the topic. Due to the small number of clinical studies, we have not performed a GRADE-analysis per outcome measure, but we chose to make the level of evidence per included study per recommendation explicit, as this may be more insightful for the interpretation of the recommendation.

Given our intention to make these guidelines applicable and able to be utilized internationally, we have crafted the recommendations to be feasible in different countries with varying health care systems. Moreover, we created a surveillance scheme to more easily review the needs at time of diagnosis and at follow up with age (supplement, surveillance scheme). In the end, consensus was reached on all statements during a face-to-face meeting with the international consortium members. The methodology used here is a solid and replicable methodology that is based on systematically searched and selected literature, professional experience, and patient representatives' experience. As such, this methodology can serve as a basis for similar future efforts for other rare mNDDs and genetic conditions.

The consortium will revisit the clinical consensus guideline every five years, to evaluate whether any recommendations have changed due to new literature or clinical insights. If no changes are applicable, the guideline statements will be re-established. If changes are required, the consortium

will propose an update for the recommendations after a systematic search has been performed and the literature has been analyzed.

Conclusion

This guideline presents 66 Kleefstra syndrome (KLEFS1)-specific, evidence-based recommendations formulated by an international KLEFS1 consortium. These recommendations aim to enhance patient care, diagnostics, awareness, and management for KLEFS1 individuals on a global scale. This guideline serves a significant need in the dissemination and application of current knowledge. Furthermore, it serves as a valuable methodological example for guideline development in the context of rare mNDDs and other disorders with limited evidence. As the clinical management is largely applicable including also in relative resource-limited settings, timely diagnosis and guided management based on the current recommendations is expected to improve quality of life of patients and relatives at a global scale.

Data Availability

All directly relevant data are included in this study. The supplement summarizes symptom prevalences. Voting results are available from the corresponding author upon request.

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Author Contributions

Conceptualization: A.B., C.S., C.G., T.K.; Data curation: A.B., C.S., C.G., T.K.; Formal analysis: A.B., C.S., C.G., T.K.; Investigation: A.B., C.G., C.S., T.Z., D.R., J.G., L.M., A.M., D.W., S.R., I.F., J.D., A.O., L.P., C.S., D.M., R.H., B.T., K.D., L.G., M.F., A.D., L.R., E.P., I.G., K.H., L.B., Z.F., M.C., K.S., L.E., S.S., N.B., E.C., A.S., R.S., N.B., S.T., S.V., H.B., M.K., K.V., ERN Ithaca, T.K. ; Methodology: A.B., C.S., C.G., T.K., Project administration: A.B.,K.V; Resources: A.B., C.S., C.G., T.K.; Software: A.B., C.G., T.Z., T.K.; Supervision: T.K.; Validation: A.B., C.S., C.G., T.K.; Visualization: A.B., C.S., C.G.; Writing original draft: A.B., C.S., C.G., T.K.; Writing review and editing: A.B., C.G., C.S., T.Z., D.R., J.G., L.M., A.M., D.W., S.R., I.F., J.D.,

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Ethics Declaration

Verbal informed consent was obtained from the parents participating in the focus group prior to each meeting, including consent for audio recording and the processing of data for the development of the guideline.

Participation in the consortium for the development of the guideline was entirely voluntary, and consent was considered to be implied through active participation.

Conflict of interest statement

All authors declare that they have no conflicts of interest.

Supplemental file

[Insert supplemental file: focus group parents and list of clinical and patient expertise centers]

[Insert supplemental file: prevalences Kleefstra syndrome]

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Figure Legends

Figure 1 The PRISMA flow diagram for new systematic reviews which included searches of databases, registers and other sources. From: *Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. Doi: 10.1136/bmj.n71*

Figure 2 Diagnostic pathway for individuals with unexplained NDD and/or suspected KLEFS1

LP/P = likely pathogenic/pathogenic (per ACMG guidelines)
VUS = variant of uncertain significance
GS/ES = genome sequencing / exome sequencing
CMA = chromosomal microarray
DNAm = DNA methylation
MEA = multielectrode array
LR= long

Table 1 Recommendations for Kleefstra syndrome type 1	**Literature**	**Literature: Level of evidence**	**Delphi procedure: Level of evidence[a]**
Genetics			
1. Refer individuals with features suggestive of Kleefstra syndrome as objectively assessed by a clinician, or when parents have raised their concerns, for appropriate evaluation and consideration of genetic testing.	*Expert opinion*	-	A
2. Perform genome sequencing (GS) (or exome sequencing (ES) if GS is not accessible), preferably as a trio, as an initial test for individuals with clinical suspicion of Kleefstra syndrome. Ideally, the genomic test (GS/ES) includes evaluation for sequence variants as well as copy number and structural variants. If GS/ES as an initial test is non-diagnostic, then perform chromosomal microarray (CMA, standard or high resolution) as the next test. If GS/ES is not accessible as a first test, perform CMA as the initial test, followed by GS/ES if CMA is non-diagnostic.	**General:** [16] [17]	5	B
3. Refer individuals with an *EHMT1* variant (pathogenic or variant of uncertain significance, VUS) to a clinical genetics team for phenotype evaluation, diagnosis confirmation, and genetic counselling for the individual/family. If a clinical genetics team is not accessible, refer the individual to a clinician specialized in genetic neurodevelopmental conditions.	**General:** [39] [40]	5	A
4. Offer standard karyotyping and FISH with probes targeting *EHMT1* for individuals with known 9q34.3 deletion and both parents, to evaluate the presence of a balanced structural variant (SV) in a parent and to estimate the chance of recurrence. Genome (long read) sequencing may replace FISH for balanced SV detection.	**General:** [34]	5	A
5. Consider gene-targeted copy number variant (CNV) analysis to detect small structural variants such as single exon CNVs in individuals with non-diagnostic GS/ES and standard CMA but with a remaining high clinical suspicion for a Kleefstra syndrome diagnosis, because they may be missed by standard GS/ES or CMA.	**KLEFS1-specific:** [1] [71]	3	A
6. Consider additional diagnostic laboratory tests (e.g., DNA methylation signature) or inclusion in research studies for individuals with phenotypic features highly suggestive of Kleefstra syndrome but with non-diagnostic testing, including VUS in *EHMT1.*	**KLEFS1-specific:** [1] [21] [88] **General:** [89]	4	A
Mental health-Behavior			
7. Review behavior and mental health profile at diagnosis of Kleefstra syndrome and annually thereafter, taking into account medication use,	**KLEFS1-specific:** [30] [31] [41] [52] [65] [66] [67] [71] [90]	4	A

co-occurring conditions, and current provision of management and support.			
8. Refer individuals for further evaluations by professionals when behaviors or mental health difficulties are identified, for diagnostic assessment (e.g. autism assessment) and/or intervention (e.g. behavioral support and/or medication as appropriate).	**KLEFS1-specific:** [1] [3] [44] [46] [49] [31] [71] [30] [41]	3	A
9. Proactively refer individuals to psychiatric/psychological care when there are symptoms suggestive of conditions that cause functional impairment, e.g. those conditions delineated in the DSM-5.	**KLEFS1-specific:** [47] [91] [65] [52] [44] [67] [46] [92] [3] [51] [71]	3	A
10. Implement personalized interventions for behaviors of concern as a primary approach, based on behavioral principles and a functional assessment.	**KLEFS1-specific:** [71] [30] [66]	4	A
11. Proactively evaluate and act upon (for example organize appropriate diagnostic review and treatment) any sudden emergence of behaviors and mental symptoms, or significant changes.	**KLEFS1-specific:** [71] [66] [65]	4	A
Regression			
12. Urgently refer individuals with (suspected) regression to an experienced clinician (psychiatrist, neurologist, pediatrician) for assessment, diagnosis (including comprehensive etiological evaluation) and treatment	**KLEFS1-specific:** [66] [92] [44] [47] [52]	4	A
13. Use instruments to measure and monitor adaptive functioning to accurately track regression	**KLEFS1-specific:** [66] [44] [47] [52]	4	A
14. Consider initiation of Olanzapine, in consultation with a psychiatrist, for new or ongoing regression, especially in the presence of psychosis and severe insomnia. Catatonia, which can also present as regression, may warrant a different treatment pathway.	**KLEFS1-specific:** [47] [52] [66] [92] (Unpublished data, Radboudumc)	4	A
15. Contact a specialized center for Kleefstra syndrome for further evaluation, management of regression, and collaborative data collection.	**KLEFS1-specific:** [52]	4	B
Neurology			
16. Consider a brain MRI at diagnosis of Kleefstra syndrome when clinically indicated, particularly when one of the following issues is present: focal seizures, focal findings on electroencephalography (EEG), developmental regression, or acute onset of psychosis/catatonia. Consider the use of Gadolinium with MRI brain as clinically indicated.	**KLEFS1-specific:** [71] [93] [36] [47] [53]	4	A
17. Consider follow-up brain MRI when there are actionable findings on a prior brain MRI or when there are clinical changes. Such clinical changes include, but are not limited to, new focal seizures, new focal findings on EEG, acute and significant worsening of seizure frequency, first episode of developmental	**KLEFS1-specific:** [71] [93] [36] [47] [53]	4	A

regression, first episode of psychosis or catatonia. Consider the use of Gadolinium with MRI brain as clinically indicated.			
18. Refer individuals to a neurologist or physician specialized in epilepsy management if there are spells concerning for seizures, or a new diagnosis of epilepsy. Treat epilepsy as per local protocol.	**KLEFS1-specific:** [2] [41] [7] [94] [95] [96] [97] [54] [98] [60]	4	A
19. Consider a routine electroencephalogram (EEG) as an initial assessment when there is concern for seizures or epilepsy, or if there is developmental regression. Include video recording and capture both awake and asleep periods. Consider a follow-up EEG for new types of spells concerning for seizures, new seizure semiologies, or significant increases in frequency of existing seizures	**KLEFS1-specific:** [41] [7] [46] [95] [97] [54] [94] [60]	4	A
20. Wean off antiseizure medications (ASMs) as per local protocol. However, consider continuing ASMs for a longer period of time if the epilepsy has been severe, difficult to control, brain MRI shows findings likely contributing to epileptogenicity (e.g., focal seizures in the setting of a focal cortical dysplasia), or based on shared decision making with the individual/family.	**KLEFS1-specific:** [54] **General:** [57]	4	A
Sleep			
21. Review sleep regularly at all ages, discuss monitoring strategies and preferences with caregivers (e.g. sleep diaries).	**KLEFS1-specific:** [46] [3] [1] [64] [31] [52] [62] [47] [99] [63] [71] **General:** [100] [68]	3	A
22. Recommend adherence to sleep hygiene strategies as outlined in guidelines for sleep in neurodevelopmental conditions. Recommend tailoring activities during the day and during the bedtime routine to an individuals' sensory profile.	**KLEFS1-specific:** [71] [64]	4	A
23. Evaluate and address somatic, psychiatric, social and environmental factors contributing to sleep problems.	**KLEFS1-specific:** [71] [64] [3] [69] [97] [67] [52] [66] [63] [1]	3	A
24. Consider proactive referral to a sleep specialist when poor sleep is reported.	**KLEFS1-specific:** [71] [74]	4	A
25. Be aware of paradoxical reactions to sleep medication (e.g. benzodiazepines, melatonin); discourage use without professional support.	**KLEFS1-specific:** [62] [66] [64]	4	A
26. Respond to acute severe changes in sleep patterns from puberty onward following guidance presented in the regression chapter.	**KLEFS1-specific:** [67] [52] [66] [63] [71] [101]	4	A
Hearing			

27. Perform audiological assessment at diagnosis of Kleefstra syndrome, annually until the age of 6 years, and thereafter every 2 years until early adolescence. Assessment can be more frequent if clinically indicated or if concerns arise from the individual/family. Pay particular attention to the presence of central auditory processing disorders.	**KLEFS1-specific:** [1] [102] [7] [3] [36] [103] [9] [65] [69]	3	A
28. Perform audiological assessment in adults following guidelines for individuals with intellectual disability.	**KLEFS1-specific:** [71] [65] [74] [3]	3	A
29. Prevent the build-up of earwax	**KLEFS1-specific:** [103]	4	A
30. Proactively manage otitis media and ear effusions early to minimize discomfort, prevent hearing loss and optimize (language) development and functioning, especially during critical developmental windows.	**KLEFS1-specific:** [3] [1]	3	A
31. Apply tailored vision and hearing interventions, taking into account developmental age, degree of sensory dysfunction, behavioral issues, sensory processing disorders and tactile defensiveness.	**KLEFS1-specific:** [3] **General:** [104]	3	A
Vision			
32. Refer to an ophthalmologist at diagnosis of Kleefstra syndrome and if there are concerns from the individual/family. Pay particular attention to strabismus, refractive errors, in particular hypermetropia, and the presence of cerebral visual impairment.	**KLEFS1-specific:** [1] [3] [71] [31] [73] [32] [65] [102] [95] [103] [105] **General:** [70]	3	A
33. Assess visual acuity regularly as per local protocols, pay particular attention to strabismus, especially in children under 6 years of age. In adults, follow international guidelines for individuals with intellectual disability.	**KLEFS1-specific:** [3] [71] [65] [74] [36] [47] [102] [47] [65] [36] [106] **General:** [107] [108] [70]	3	A
34. Manage vision conditions as per local protocol, refer to a clinical specialist (e.g. low vision rehabilitation specialist, occupational therapist, psychologist) as needed for characterizing visual challenges and suggesting adaptive strategies and assistive (technological) devices.	**KLEFS1-specific:** [3] [74] [65] [71] **General:** [109]	3	A
Speech-Language-Communication			
35. Assess speech and language at diagnosis of Kleefstra syndrome, and then at least annually until age 12, conducted by a speech therapist or logopedics.	**KLEFS1-specific:** [71] [31] [110] [36] [111] [112] [76] [52] [95] [72] [55] [77, 96] [65] [32] [75] [7] [73] [41] [74] [113] [114] [98] [115] [3]	3	A
36. Assess speech and language in adolescents and adults as needed, e.g., if concerns arise from the family or after the acute phase of a regressive episode, to set therapy goals and to monitor change.	**KLEFS1-specific:** [65] [7] [41] [74] [67] [106] [13] [47] [9] [91] [99] [62]	3	A

	[116] [117] [44] [66] [3] [71]		
37. Perform Augmentative and Alternative Communication (AAC) evaluation if individuals cannot use speech to meet all their everyday communication needs.	**KLEFS1-specific:** [13] [55] [74] [62] [114] [47] [99] [65] [36] [111] [52] [95] [72] [77] [75] [73] [2] [98] [113] [116] [117] [106] [9] [112] [76] [32] [115] [3] [44]	3	A
38. Provide therapy, tailored to an individual's specific speech and language diagnoses, at diagnosis of Kleefstra syndrome and regularly thereafter, covering speech, language, and AAC including implementing sign and gesture if indicated.	**KLEFS1-specific:** [3] [62] [52] [110] [67] [31] [77] [78] [71]	3	A
39. Adapt AAC to individual's specific communication, cognitive, vision, hearing and co-occurring neurodevelopmental conditions, such as autism and sensory processing disorder.	**KLEFS1-specific:** [71]	5	A
Cardiology			
40. Refer to a cardiologist at diagnosis of Kleefstra syndrome, for evaluation of congenital heart defects with an echocardiogram or cardiac MRI, and for rhythm abnormalities with an electrocardiogram (ECG).	**KLEFS1-specific:** [71] [83]	5	A
41. Consult with a cardiologist when commencing new medications with potential cardiac implications (e.g. psychiatric, prokinetic and certain neurological medications)	**KLEFS1-specific:** [1] [41] [7] [83]	3	A
42. Manage structural heart defects and/or rhythm abnormalities as per local protocol.	**KLEFS1-specific:** [83]	4	A
43. Maintain a low threshold for ECG monitoring. Starting from age 18, perform an annual screening ECG in all individuals with Kleefstra syndrome to evaluate for rhythm abnormalities	**KLEFS1-specific:** [7] [83] [71]	4	B
44. Urgently refer individuals to a cardiologist for further evaluation if there are abnormalities on the ECG, or if new or worsening cardiorespiratory symptoms develop	**KLEFS1-specific:** [41]	4	A
45. Consider repeating imaging, ECGs, and/or longer-term ambulatory rhythm monitoring in individuals with new or worsening cardiorespiratory symptoms or unexplained decline in neurocognition, mood, or functional status.	*Expert opinion*	-	A
Growth-Metabolism			

46. In childhood and adolescence, measure height and weight regularly and assess growth using appropriate charts and target-height range comparison in children and adolescents. Measure head circumference regularly and assess using appropriate charts in children at least until age 3. In adulthood, measure and assess weight, waist circumference, and BMI at least annually or upon clinical indication.	**KLEFS1-specific:** [48]	3	A
47. Perform a diagnostic work-up if short stature and/or overweight is present as per local protocol (e.g. evaluation of growth hormone, thyroid function and bone age) to detect possible treatable causes.	**KLEFS1-specific:** [48]	3	A
48. Offer guidance on a healthy lifestyle, including physical activity and healthy eating habits. Consider referring individuals with Kleefstra syndrome to a dietitian/nutritionist, especially if they are overweight/obese or have risk factors such as being on psychotropic medications (see Chapter mental health and behavior)	**KLEFS1-specific:** [48] [67]	4	A
49. Evaluate thyroid function at least at puberty onset and upon clinical indication. Monitor for (pre)diabetes in adolescents and adults as per local protocol.	**KLEFS1-specific:** [48] [76]	3	A
50. Evaluate bone density at puberty onset and when multiple or unexplained fractures occur as per local protocol. Consider Vitamin D supplementation, especially when there is limited exposure to sunlight.	**KLEFS1-specific:** [48]	3	A
Constipation			
51. Evaluate for constipation at all ages by a careful history, abdominal, perineal, and if necessary rectal digital examination.	**KLEFS1-specific:** [13] [1] [3] [116] [46] [31] [66] [98] [7] [65] [42] **General:** [118]	4	A
52. Be aware and inquire about the possible occurrence and worsening of existing constipation (which might have an atypical presentation), especially if there is an increase in behavioral, sleep or psychiatric issues.	**KLEFS1-specific:** [65] **General:** [118]	4	A
53. Be aware of concerning symptoms which may suggest an additional underlying factor for the constipation. Consider diagnostic laboratory tests, including TSH and fT4.	**KLEFS1-specific:** [48] [76] **General:** [118]	4	A
54. Recommend increased fluid and fiber intake, physical activity and healthy toileting behaviors to prevent and treat constipation.	**KLEFS1-specific:** [48] **General:** [118]	4	A
55. Prescribe treatment for constipation as per local protocol, unless there is a risk of aspiration.	**General:** [118]	5	A
56. Refer individuals to a gastroenterologist if constipation persists despite regular therapy.	**KLEFS1-specific:** [31] [65] [66] [98] [7]	4	A

	General: [118]		
Parental support			
57. Offer timely clear, honest and accessible explanations about the cause of the condition, information regarding appropriate health checks according to the current guideline and the natural history of the condition.	**KLEFS1-specific:** [46] **General:** [119] [120]	4	A
58. Provide information aligning to the preferences of an individual/family, main concerns, and coping abilities. Offer follow up appointments to discuss any questions that individuals, families and support people have across the lifespan.	**KLEFS1-specific:** [46] [45] [66] **General:** [121]	4	A
59. Provide individuals/families and support people with details of Kleefstra syndrome patient advocacy/peer-support organizations, counselling services, and reliable sources of information.	**KLEFS1-specific:** [46] [45] **General:** [121] [122] [123]	4	A
60. Enquire about the emotional wellbeing of individuals/families (including siblings and grandparents) at all appointments and offer appropriate support.	**KLEFS1-specific:** [46] [45] **General:** [124] [125] [126] [127] [121]	4	A
61. Consult with experts and expert centers about Kleefstra syndrome, if required.	**KLEFS1-specific:** [46] **General:** [128] [121]	4	A
62. Be open to the information that individual/family and support providers bring to appointments.	*Expert opinion* **General:** [129]	-	A
63. Link the individual/family with available opportunities to participate in data registries and natural history studies.	**KLEFS1-specific:** [46] **General:** [128] [121]	4	A
64. Support individuals/families to access relevant support services, such as financial assistance, educational resources, disability supports, and respite services.	**KLEFS1-specific:** [46] [130] **General:** [124] [125] [126] [127] [131] [132] [133] [134] [121]	4	A
65. Discuss a transition to adult care plan, proactively starting in mid-adolescence.	**General:** [135] [136] [137]	5	A
66. Link individuals/families where possible to a multidisciplinary clinic and/or care coordinator.	**General:** [138] [139] [140] [141] [121]	5	A

[a]A: complete agreement (100%), B: high agreement (95-99,9%), C: good agreement (90-94,9%), D: weak agreement (80-89,9%), E: very weak agreement (70-79,9%)