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ALG13-Congenital Disorder of Glycosylation (ALG13-CDG): Updated Clinical and Molecular Review and Clinical Management Guidelines

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Abstract

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Author Contributions

RS, RA, and EM were involved in the conceptualization and wrote the manuscript. Visualization: RS and IS. All data collection: RS and RA. Variant data collection: IS. Neurological data collection: MS, EE. Glycosylation and enzymatic data collection: SR. Skeletal Data Collection: SV. Manuscript editing; clinical section: RS, EE, RA, and AE; diagnostic section: HHF, BGN, TK, and MH. Funding acquisition: TK and EM. All authors were involved in the interpretation of the collected data and editing the manuscripts. All authors have read and approved the manuscript.

Conflict of interest

All authors report no conflict of interest.

ALG13-Congenital Disorder of Glycosylation (CDG), is a rare X-linked CDG caused by pathogenic variants in ALG13 (OMIM 300776) that affects the N-linked glycosylation pathway. Affected individuals present with a predominantly neurological manifestation during infancy. Epileptic spasms are a common presenting symptom of ALG13-CDG. Other common phenotypes include developmental delay, seizures, intellectual disability, microcephaly, and hypotonia. Current management of ALG13-CDG is targeted to address patients' symptoms. To date, less than 100 individuals have been reported with ALG13-CDG.

In this article, an international group of experts in CDG reviewed all reported individuals affected with ALG13-CDG and suggested diagnostic and management guidelines for ALG13-CDG. The guidelines are based on the best available data and expert opinion. Neurological symptoms dominate the phenotype of ALG13-CDG where epileptic spasm is confirmed to be the most common presenting symptom of ALG13-CDG in association with hypotonia and developmental delay. We propose that ACTH/prednisolone treatment should be trialed first, followed by vigabatrin, however ketogenic diet has been shown to have promising results in ALG13-CDG. In order to optimize medical management, we also suggest early cardiac, gastrointestinal, skeletal, and behavioral assessments in affected patients.

Keywords

ALG13-CDG; Congenital disorders of glycosylation; X-linked CDG; epileptic spasm; Seizure disorder

Introduction

ALG13-Congenital Disorder of Glycosylation (CDG) is an X-linked CDG that affects both males and females and occurs due to *de novo* or inherited missense variants in the asparagine-linked glycosylation 13 (*ALG13)* gene[1]. ALG13's glycotransferase activity is essential for the protein N-linked glycosylation pathway, adding the second N-acetylglucosamine sugar onto the growing glycan chain of the dolichol-P-P precursor[2]. ALG13 forms a heterodimer with ALG14, which anchors ALG13 to the ER membrane, but ALG14 does not have glycotransferase activity[3–5].

There are different isoforms of the ALG13 protein and *in vitro* assays have shown that only isoform 2 plays a role in the N-glycosylation pathway [6]. Isoform 2, which is the smallest isoform, is composed of one domain known as the glycosyltransferase 28 domain, which carries out the aforementioned essential function in the protein N-glycosylation pathway. Thus, we define ALG13-CDG when there are pathogenic variants in the glycosyltransferase 28 domain[7]. These missense variants have been shown to have less than 28% residual ALG13 glycosyltransferase activity [8]. The most common *ALG13* pathogenic variant is c.320A>G (p.N107S) which severely impacts ALG13 *in vitro* enzymatic activity, reducing it to only 8% of control. Despite a significant decrease in enzymatic activity, the plasma transferrin glycosylation profiles and N-glycan profile of other serum glycoproteins of essentially all females and the majority of males with ALG13-CDG are normal or near normal, showing that a defect in ALG13 glycosyltransferase function does not lead to secretory glycosylation abnormalities. The lack of glycosylation abnormalities in the serum

glycoproteins of almost all individuals with ALG13-CDG adds to the complexity of diagnosing this disorder [9,10]. Thus, ALG13-CDG is caused by pathogenic variants in the glycosyltransferase 28 domain (125 Amino Acids in length) as confirmed by *in vitro* enzymatic assay[8], even without serum glycosylation abnormalities.

Currently, there are 83 reported individuals with confirmed pathogenic variants in the glycosyltransferase 28 domain. Neurological symptoms dominate the phenotype of ALG13-CDG with the majority of affected individuals displaying seizures and global developmental delay as the first symptoms[11]. Current treatment options for ALG13-CDG are limited to anti-seizure medication (ASM) and ketogenic diet to control seizures. In this study, we review the clinical, biochemical, and molecular data for all previously reported individuals, and we provide a detailed guideline on the diagnostic approach to ALG13-CDG, evaluation for multi-systems involvement, and symptomatic treatment strategies.

Methodology

These guidelines were developed by an international group of experts in CDG who reviewed all the relevant literature since 2012, the year in which ALG13-CDG was first identified until December 2023. A PubMed database search was performed via the utilization of keywords *ALG13*, ALG13-CDG, and developmental and epileptic encephalopathy 36. Any variants outside the GTS28 domain, encoded by the proximal *ALG13* exons which are responsible for ALG13 glycosyltransferase activity are excluded from this review. A literature review and clinical, biochemical, and molecular data from all previous reports were collected and analyzed. This is followed by developing a consensus regarding diagnosis, follow-up, and management of ALG13-CDG. For the most part, the evidence and resulting recommendations are considered experts' opinions because additional levels of evidence were not available in the literature. Evidence grading was classified in accordance with the Scottish Intercollegiate Guidelines Network (SIGN) methodology (http://www.sign.ac.uk). Supplementary file1

Results

A total of 66 articles were reviewed. Out of the 66 articles, 32 were in vitro studies, 15 were case reports, 16 were systemic reviews, and 3 were in vivo studies[1,6–7,9–31].

All individuals with *ALG13* genetic variants in the glycosyltransferase 28 domain were reviewed and those with distal missense variants in ALG13 were excluded. Distal missense variants are not expected to impact glycosylation and there are no functional methods to prove the pathogenicity of these variants.

In total, we identified 83 individuals with variants in the glycosyltransferase 28 domain all of which were confirmed pathogenic variants (Figure 1).

General clinical data

A total of 83 individuals (4 males and 79 females) with ALG13-CDG were included in this review. Clinical data was available for 80 of these individuals and their ages ranged

from 3.5 months to 29 years (median age was 4 years). The age of symptoms onset for these individuals ranged from birth to 9 months. During early infancy, many of these individuals displayed epileptic spasms with concomitant hypotonia and motor delay. Figure 2 summarizes the multi-system involvement in individuals with ALG13-CDG.

Neurological phenotype

Neurological involvement predominates the phenotype of ALG13-CDG. Almost all the individuals with ALG13-CDG presented with at least two neurological phenotypes. Global developmental delay was diagnosed in 72 individuals. Two or more developmental domains were affected, mainly motor function (n=72), cognition (n=70), and speech (n=34). Most individuals with ALG13-CDG cannot communicate verbally. However, most individuals learn to walk and are mobile when they get older (by the age of 4 to 5 years). Hypotonia was reported in two-thirds of the individuals (n=56), and movement disorders were seen in 22 individuals, including choreoathetosis (n=8), ataxia (n=4), dystonia (n=6), and stereotypic movement (n=10).

Seizure was the most prevalent feature in ALG13-CDG. A total of 79 individuals were reported to have experienced seizures. The majority (n=65, 82 %) were described to have epileptic spasms (ES) as an early finding, with a mean age at the first seizure onset of 5.7 months (median 5.0, range 1–24). Outcome data exist for 63 of these individuals where 26 (41%) later became seizure-free, either on (n=13) or off (n=13) antiseizure medication (ASMs) or on ketogenic diet (n=25). The rest had either persistent or relapsing epileptic spasms (n=9, 14%), or developed different seizures semiology including generalized seizure (tonic, tonic-clonic, absences, atonic, myoclonic, n=16), Lennox-Gastaut syndrome (n=8) or focal seizures (n=5). Seizure type was not specified in five individuals.

There was variability in the initial findings on the electroencephalographic (EEG) recordings, where the most common finding was hypsarrhythmia (n=52). In addition, some of these individuals had a multifocal/generalized pattern, but not a fully developed hypsarrhythmia (n=3), abnormal bilateral slow-wave activities (n=1), or a focal disturbance (n=2). Multiple therapies were used to control the seizures. To treat the initial IS, ACTH or prednisolone was used in 36 individuals, where there often was an initial effect, however, only few individuals remained seizure-free in the long term. In 21 of these individuals, vigabatrin (VGB) was used in the initial treatment and there was an initial response in about half of them, but only a few stayed seizure-free. Some of these individuals also showed an improvement on ketogenic diet (n=12). Almost all of these individuals received other ASMs after the epileptic spasms had resolved or relapsed, and despite ASMs their seizures were refractory. Most commonly used ASMs were (VGB, n=26), valproic acid (VPA, n=22), topiramate (TPM, n=21), levetiracetam (LEV, n=25), and benzodiazepines (n=22). Other common ASMs were also used to some degree. Given the retrospective approach of these recommendations, it was hard to determine which ASMs were most effective. ALG13-CDG individuals often show pronounced refractory seizures.

Microcephaly was the most common abnormality seen in individuals with ALG13-CDG (n=15). Macrocephaly was reported in only one individual. The most common brain MRI finding was cerebral atrophy (n=18), followed by delayed myelination (n=7). Other rarer

brain MRI findings include thinning of the corpus callosum (CC) (n=2), large cisterna magna (n=1), and hydrocephalus (n=1).

Presentation (Statement #1: grade of recommendation C): The initial presentation of ALG13-CDG is most typically with an epileptic spasm and hypotonia. The main neurological phenotypes in ALG13-CDG are developmental delay, seizures, intellectual disability, hypotonia, microcephaly, and movement disorders. EEG for many of the affected individuals display hypsarrhythmia.

Diagnosis and follow-up (Statement #2: grade of recommendation C): At the time of ALG13-CDG diagnosis, full neurological assessment, EEG, and neuroimaging should be done and yearly follow-up should be done by a neurologist.

Treatment (Statement #3: grade of recommendation C): As seizures have an immense impact on the quality of life of individuals with ALG13-CDG, they should be on antiseizure medication. ACTH, prednisolone, and vigabatrin can be used for epileptic spasms[32]. However, ACTH/prednisolone probably should be trialed first (ref.). Ketogenic diet has been shown to be promising in ALG13-CDG. No ASM stands alone as first line treatment after the initial epileptic spasms have resolved or developed into other seizure types. ASM therapy should be managed on an individual basis to minimize side effects and optimize clinical outcomes.

Behavioral abnormalities

A total of 18 individuals with ALG13-CDG reported one or more behavioral abnormalities including autism (n=9), self-mutilation (n=3), sleep difficulties (n=7), and aggression (n=2). The majority of individuals with ALG13-CDG are either non-verbal or only speak a few words.

Presentation (statement #1: grade of recommendation C): Individuals with ALG13-CDG can display autism and sleep difficulties.

Diagnosis and follow-up (statement #2: grade of recommendation D): Behavior abnormalities should be evaluated as a standard of care for ALG13-CDG at diagnosis and annually thereafter

Management (statement #3: grade of recommendation D): There are no ALG13-CDG specific management strategies for the behavioral abnormalities. Standard supportive treatments such as behavioral management therapy and psychological referral are recommended.

Ophthalmological phenotype

Ophthalmologic involvement has been reported in 30 individuals with ALG13-CDG. Cortical visual impairment was the most common ophthalmologic abnormality, (n=6), followed by strabismus (n=4), astigmatism and hyperopia (n=2), nystagmus (n=2), and delayed visual maturation (n=1). The type of strabismus was only specified for one of the

individuals as binocular. Ophthalmological abnormalities in 15 of the individuals were not specified.

Presentation (statement #1: grade of recommendation C): Individuals with ALG13-CDG have vision impairment due to cortical visual impairment or blindness. Other abnormalities include strabismus, astigmatism, hyperopia, and nystagmus.

Diagnosis and follow-up (statement #2: grade of recommendation D): Since vision can be impacted in ALG13-CDG due to eye abnormalities and cortical abnormalities, thorough ophthalmologic assessment is recommended annually as a standard of care for individuals with ALG13-CDG.

Management (statement #3: grade of recommendation D): There are no ALG13-CDG specific management strategies for ophthalmologic abnormalities. Standard supportive treatments are recommended for strabismus, astigmatism, hyperopia, etc. Further, individuals with cortical visual impairment could benefit from early intervention and vision rehabilitation.

Skeletal phenotype

Skeletal abnormalities have been reported in 19 individuals with ALG13-CDG as part of a multisystem phenotype. Of these, 9 have scoliosis, 4 have joint laxity, 3 have osteopenia, and 3 have skeletal abnormalities that are not specified. Other, more uncommon skeletal findings that have been described in ALG13-CDG include joint contractures, lordosis, vertebral anomalies (hemivertebra), plagiocephaly, syndactyly, and adducted thumbs.

Presentation (statement #1: grade of recommendation C): ALG13-CDG can present with skeletal involvement, including scoliosis, joint laxity, and osteopenia.

Diagnosis and follow-up (statement #2: grade of recommendation D): At diagnosis, skeletal survey should be made to assess skeletal involvement. Follow-up investigation should include a regular clinical assessment of scoliosis and possible joint abnormalities. When individuals with ALG13-CDG have severe spinal deformities, pulmonary function should be tested.

Management (statement #3: grade of recommendation D): There is no disorder-specific management for skeletal abnormalities in ALG13-CDG. Unlike other CDG, there are minimal bleeding risks involved in ALG13-CDG, so scoliosis surgery does not include any additional risk if the patient’s coagulation function is normal. Scoliosis in individuals with ALG13-CDG should be treated with standard of care and regular monitoring. Braces or surgery can be appropriate depending on the severity of the scoliosis. For joint pain, individuals can be referred to a rheumatologist if necessary.

Gastrointestinal phenotype

Twenty-three individuals with ALG13-CDG were reported with gastrointestinal involvement. This is mostly attributed to hypotonia. Gastroesophageal reflux and vomiting

(n=14) are the most common symptoms followed by feeding difficulties (n=6), G-tube requirement in three, and constipation (n=5). Crohn's disease has been reported in one individual.

Presentation (statement #1: grade of recommendation C): The main gastrointestinal abnormalities in ALG13-CDG include feeding difficulties, gastroesophageal reflux, and constipation.

Diagnosis and follow-up (statement #2: grade of recommendation D): Body mass index and electrolytes should be measured at the time of diagnosis and annually thereafter. GE reflux and swallowing should be evaluated.

Management (statement #3: grade of recommendation D): Nutritional support and feeding support should be provided to all ALG13-CDG individuals with GI involvement. Anti-reflux measures should be taken after eating. Oral motor therapy should be used in ALG13-CDG individuals with feeding difficulties.

Cardiac phenotype

Cardiac involvement has been reported in 7 individuals with ALG13-CDG. This involvement includes arrhythmia (n=2), congenital heart defects (n=2), bradycardia (n=1), mild pulmonary stenosis (n=1), and mitral valve regurgitation (n=1).

Less commonly reported systems involvement (immunological, respiratory, endocrine, hematological, and hearing)

Immunological abnormalities were reported in one cohort (n=5/26), but details were not specified. There has not yet been a thorough description of the immunological involvement in ALG13-CDG. However, atypical and recurrent infections have been reported in other CDG and ALG13-CDG cases. Recurrent infections have been described in 2 male individuals with ALG13-CDG, one of which was a respiratory infection. No further immunological work-up was performed in those cases [20,21]. Respiratory involvement was reported in the same cohort (n=5/26) but not further specified. There were no reported endocrine abnormalities in ALG13-CDG individuals. Hematological abnormalities were reported in 3 individuals: Elevated ATIII, protein C, increased bleeding tendency, and prolonged APPT. Factor IX and XI were both normal in these individuals. Hearing loss has been reported in three individuals with ALG13-CDG, two of them presented with unilateral sensorineural hearing loss, and one presented with asymmetrical hearing loss.

Statement #1 (grade of recommendation: D)—Echocardiography, thyroid function tests, coagulation profiles, and hearing should be screened in individuals with ALG13-CDG at the diagnosis and followed accordingly. Screening for immunological dysfunction is recommended in case of atypical and recurrent infections.

ALG13-CDG diagnosis

If ALG13-CDG is suspected, it should be evaluated by genetic testing. Exome, genome, and targeted sequencing can be performed to identify variants in *ALG13*. Individuals

with *ALG13* variants in the glycosyltransferase 28domain can be tested for glycosylation abnormalities via transferrin isoelectric focusing (TIEF) or mass-spectrometry-based methods for carbohydrate-deficient transferrin or plasms semi-quantitative N-glycan profiling however, these are likely to be normal, especially in females. Currently, there are no biochemical tests to confirm ALG13-CDG.

Molecular diagnosis

Genetic testing—ALG13-CDG is diagnosed based on genetic tests such as targeted sequencing, exome (WES), and genome sequencing (WGS). *ALG13* is located on chromosome X and contains 27 exons. So far, eight different variants in *ALG13* have been reported spanning across the glycosyltransferase domain of *ALG13*, which are associated with ALG13-CDG (Figure 1). The most common variant is c.320A>G (p.N107S) (n=73), followed by c.241G>A (p.A81T) (n=4). Seven variants are missense, while one deletion variant has been reported (c.207_209del AGA). The majority of the variants (75) occurred *de novo*, however, familial incidence was also reported[14]. There are only 4 males identified with ALG13-CDG to date with the GTS28 Domain variants.

Statement #1 (grade of recommendation: B): Genetic testing is the main method used to confirm a clinical diagnosis of ALG13-CDG.

Biochemical diagnosis

Glycosylation studies—Glycosylation abnormalities in suspected CDG can be assessed in blood by methods including transferrin isoelectric focusing (TIEF) and mass-spectrometry-based methods for carbohydrate-deficient transferrin or semi-quantitative N-glycan profiling of total plasma glycoproteins, which have previously been described in detail [9,50,51,68,69]. Likewise, glycosylation abnormalities couldbe assessed in individuals with variants in *ALG13*. Slightly abnormal glycosylation has been found in only 4/83 reported individuals including increased asialo and disialo transferrin suggestive of CDG type I[21], borderline CDG type I[9], mild undergalactosylation[9] and mildly decreased glycosylation[20], and the majority of the 83 cases had at least one TIEF test. Normal glycosylation in blood is much more common than abnormal results and does not rule out ALG13-CDG diagnosis.

Pre-analytical requirements for biochemical testing: Detailed statements on transferrin analysis sensitivity and the pre-analytical requirements for the biochemical testing are given in the PMM2-CDG guidelines[50]

Statement #1 (grade of recommendation: D): Assessment of glycosylation status in blood can be done in individuals with *ALG13* variants in glycosyltransferase domain, but are likely to be normal, which does not rule out ALG13-CDG.

Enzymatic activity assay—ALG13 forms a heterodimer with ALG14 to form a functional enzymatic complex that catalyzes the second step in lipid-linked oligosaccharide (LLO): transfer of GlcNAc from UDP-GlcNAc to GlcNAc1-PP-dolichol to form GlcNAc2-PP-dolichol[21]. ALG13/14 enzymatic activity was measured in the fibroblasts of the first

individual ALG13-CDG[21], by incubating fibroblasts with non-radioactively labeled UDP-GlcNAc and [^{14}C] GlcNAc1-PP-dolichol as an acceptor. Fibroblasts from the single *ALG13* case showed significantly reduced elongation [^{14}C] GlcNAc2-PP-dolichol (17%) compared to healthy fibroblasts. Further, an in-vitor assay to test ALG13 enzymatic assay was developed and found that pathogenic variants in ALG13 lead to more than 72% reduction in activity[8]. However, there is currently no clinically approved test for ALG13/ALG14 enzyme activity. There is no information in the literature regarding male vs female enzymatic activity.

Conclusion

Pathogenic variants in the glycosyltransferase 28 domain of the asparagine-linked glycosylation 13 (A*LG13*) affect a critical step in endoplasmic reticulum N-linked glycosylation. Like in other CDG types, multisystem involvement due to the defective N-glycosylation process is expected in ALG13-CDG individuals. These symptoms are frequently due to primary central nervous system involvement with highly variable multi-system involvement across other organ systems. Unfortunately, there are gaps in the published literature about details of the clinical phenotype of the disease as most of the reviewed literature was focused on seizure as a predominant presentation. In addition to the neurological assessment, early screening for other multi-system involvement in all CDG types including ALG13-CDG is highly recommended to provide the opportunity for early intervention and optimal management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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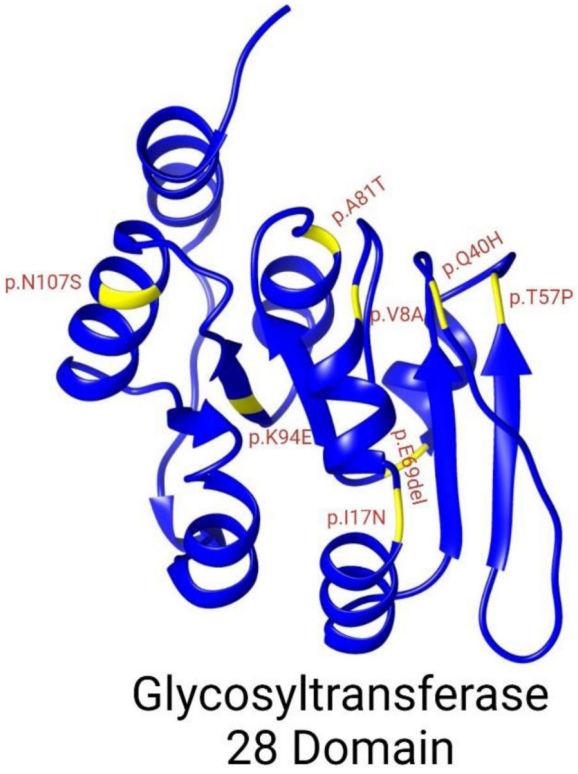

Figure 1:
AlphaFold Structure of the glycosyltransferase 28 (GTS28) domain of the ALG13 protein. Pathogenic variants in the GTS28 domain reduce its glycotransferase activity and lead to ALG13-CDG. Confirmed pathogenic variants are listed.

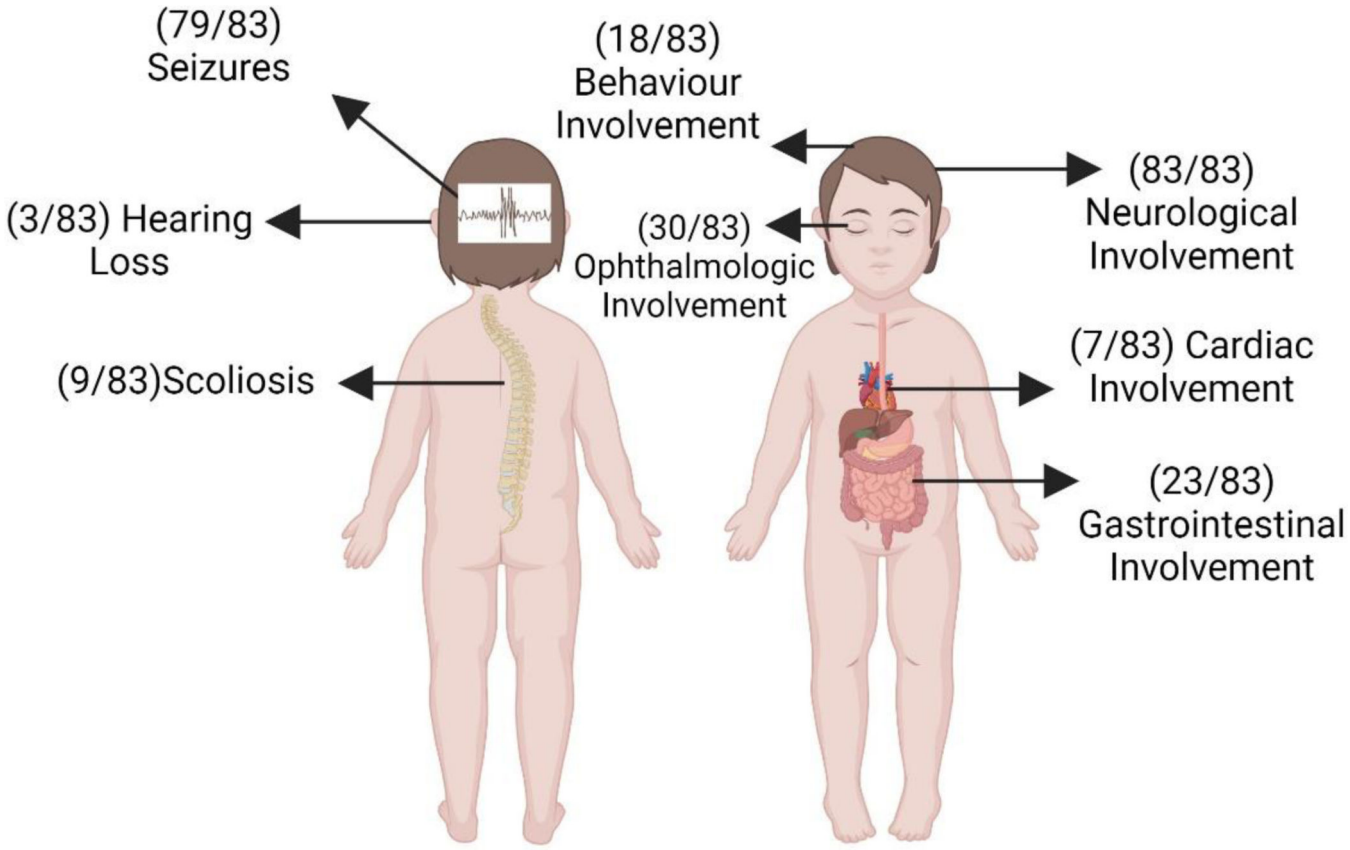

Figure 2:
Summary of System's Involvement in ALG13-CDG