SRD5A3-CDG

Current State of Research & Development 2021

CURE SRD5A3

Kristin Kantautas, PhD, Research Program Director

Omid Karkouti, MS, Research Partner

Vijay Sappani, Founder

SAPPANI FOUNDATION

Document Summary

Cure SRD5A3 is community of expert researchers, medical professionals and industry leaders working towards developing life-altering therapies and advance research on the congenital disorder of glycosylation SRD5A3-CDG.

The purpose of this document is to educate patients, caregivers, healthcare providers and researchers on SRD5A3-CDG and to provide a comprehensive overview of the current and future research efforts in the Cure SRD5A3 research program. This document is divided into four sections:

I. Cure SRD5A3 Roadmap 2021

The roadmap is an overview of the path towards therapy development for SRD5A3-CDG and Cure SRD5A3 research priorities. It provides a summary of what we currently know about each step, what information and resources we currently have and what we need to succeed in developing a therapy.

II. SRD5A3-CDG Fact Sheet

The fact sheet is a condensed summary of important facts about SRD5A3-CDG.

III. SRD5A3-CDG Lay Review

The lay review is intended to provide an overview of SRD5A3-CDG for patients, families, caregivers and members of the general public. Topics covered include an overview of congenital disorders of glycosylation, clinical features, diagnosis, mechanism of disease, ongoing clinical studies, research models used to study SRD5A3-CDG and therapeutic strategies for this condition.

IV. SRD5A3-CDG Detailed Review

The detailed review is intended for researchers, healthcare and industry professionals. This section includes a comprehensive summary of the biology of SRD5A3-CDG, the current state of research, therapeutic strategies currently under investigation in the Cure SRD5A3 program and new avenues for therapy development and translational research. **Cure SRD5A3 is currently seeking to partner with researchers, biotech and pharmaceutical companies to explore new therapeutic strategies and answer critical research questions outlined in this section.**

Contact Us:

If someone you know has recently been diagnosed with SRD5A3-CDG or are interested partnering with us, we would like to hear from you!

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Website: http://curesrd5a3.com

Email: research@sappani.com

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Cure SRD5A3 2021 Roadmap

1 DISEASE

What we know

Number of Patients: 60 reported, 10 (in contact)
Phenotype: Congenital Disorder of Glycosylation, Kahrizi Syndrome
Clinical Presentation: intellectual disability, poor muscle tone and coordination visual impairment, ocular abnormalities, skin lesions
Genotype: 15 variants reported, p.W19X is common

What we have

CDG Natural History Study (NCT04199000), SRD5A3-CDG Clincal Spectrum Study
Genotype reports & partial medical history (10 patients)

What we need

Patient registry (in progress)
Complete medical records for all reported patients
Better Understand of Disease: disease mechanism (polyprenol accumulation and/or dolichol deficiency), characterization of pathway metabolites, glycosylation defects, study pathway regulation

2 TARGET

What we know

Direct Target: SRD5A3
Indirect Target: poylprenol accumulation, dolichol deficiency, DOLK
W19X mutant SRD5A3: truncated protein, predicted complete loss-of-function

What we have

Recombinant SRD5A3 and SRD5A3 W19X cDNAs
AAV9 vectors for SRD5A3 gene therapy

What we need

Identify downstream/upstream targets
Understanding of disease mechanism

3 DRUG CANDIDATES

What we know

Therapies are non-existent. Cure SRD5A3 is exploring multiple therapeutic approaches:
Small Molecules : Statins, TRIDs
Supplementation : Sugars, terpenes, cholesterol
Investigational : Gene therapy, gene editing, *(ASOs not feasible)*

What we have

Preliminary hits from drug repurposing screen in worms
Gene therapy evaluation in cerebellum-specific *Srd5a3* KO mouse (ongoing)

What we need

Characterization of fibroblasts: pathway metabolites, gene expression and glycosylation defects (metabolomics/lipidomics, glyoroteomics, RNA sequencing)
HTS in fibroblasts: TRIDs, sugars & terpenes libraries, pathway-specific compounds
Explore investigational therapies : gene therapy for eye, gene editing, RNA editing

4 IDENTIFY LEADS

What we know

KO mouse
embryonic lethal, ↑ polyprenol:dolichol, ↑ mevalonate pathway genes

Cerebellum-specific KO mouse
ataxia, N-glycosylation defect

KO worm
slow motility, ER stress

Patient-derived cells
↑ polyprenol:dolichol

KO zebrafish
no phenotype

What we have

Cerebellum-specific condtional *Srd5a3* KO mouse
Conditional ready floxed *Srd5a3* KO mouse
Srd5a3 KO worms
Patient-derived fibroblasts

What we need

Photoreceptor-specific conditional KO mouse model, iPSCs
Biomarkers
Clinically-relevant assays for drug testing in disease models

5 PATH TO CLINICAL TRIAL

What we know

N-glycosylation defect is variable across SRD5A3-CDG patients and over time (by transferrin analysis)

What we have

Ongoing CDG Natural History Study

What we need

Natural history of disease
Clinical endpoints and outcome measures
Biomarkers

SRD5A3-CDG Fact Sheet

Lay Overview

Steroid 5a-reductase type 3 congenital disorder of glycosylation (SRD5A3-CDG, also known as CDG-Iq) is an ultra-rare genetic disorder which results in developmental delays and problems with vision. SRD5A3-CDG disrupts the normal patterns of a critical biological process called glycosylation, in which sugar groups, called glycans, are attached to proteins or fats. The SRD5A3 gene encodes polyprenol reductase, an enzyme that converts polyprenol to dolichol. SRD5A3-CDG is a promising candidate for a variety of therapeutic avenues ranging from dietary supplementation to gene therapy.

Frequency

At least 38 genetically-confirmed patients (from 26 families) have been reported in the literature.

Disorders Associated with *SRD5A3* Mutations

Congenital disorder of glycosylation 1Q (CDG1Q) [MIM:612379]

A congenital disorder of glycosylation. A multisystem disorder caused by a defect in glycoprotein biosynthesis and characterized by hypoglycosylated serum glycoproteins. Congenital disorders of glycosylation result in a wide variety of clinical features, such as defects in the nervous system development, psychomotor retardation, dysmorphic features, hypotonia, coagulation disorders, and immunodeficiency. The broad spectrum of features reflects the critical role of N-glycoproteins during embryonic development, differentiation, and maintenance of cell functions.

Kahrizi syndrome (KHRZ) [MIM:612713]

An autosomal recessive neurodevelopmental disorder characterized by mental retardation, cataracts, coloboma, kyphosis, and coarse facial features.

Gene Function

Steroid 5a-reductase type 3 (SRD5A3) encodes an enzyme called polyprenol reductase which converts polyprenol to dolichol – a critical step in protein N-glycosylation. Dolichol is required for the synthesis of dolichol-linked monosaccharides and the oligosaccharide precursor used for N-glycosylation. Polyprenol reductase promotes the reduction of the alpha-isoprene unit of polyprenol into dolichol in a NADPH-dependent mechanism. SRD5A3 is also able to convert testosterone (T) into 5-alpha-dihydrotestosterone (DHT).

SRD5A3 Gene and Variants

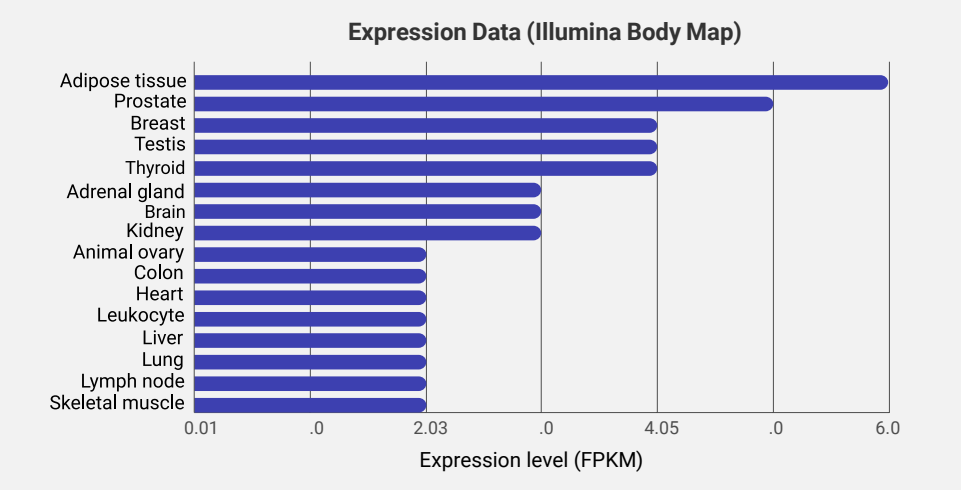
The human *SRD5A3* gene locus is located on chromosome 4p12 and contains five exons encoding a protein of 318 amino acids.

At least 15 variants in SRD5A3-CDG have been reported: 11 nonsense variants, 3 missense variants, and a large deletion.

Gene	mRNA	Protein
chr4: 55,346,221-55,373,100 (GRCh38/ hg38)length: 26,880 bases chr4: 56,212,276-56,239,267 (in GRCh37/hg19) length: 26,992 bases Orientation: Plus strand	NM_024592, 4061 bp	Symbol: Q9H8P0 Accession: Q9H8P0 2' Accessions: Q4W5Q6 Length: 318 amino acids Molecular mass: 36,521 Da

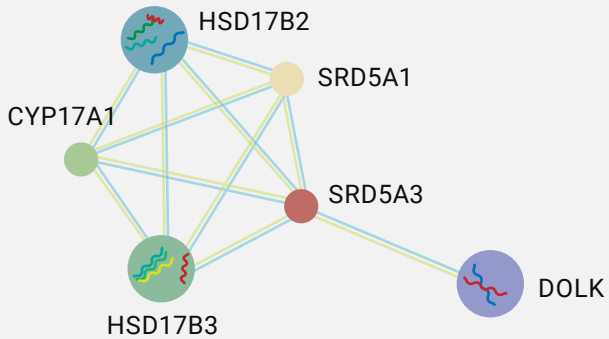
Gene Expression Profile

SRD5A3 is expressed in most human tissues including the prostate, breast, testis, thyroid, adrenal gland, brain, kidney, colon, liver, heart, lung and skeletal muscle.



SRD5A3 Protein

SRD5A3 is comprised of six transmembrane domains however the protein structure has yet to be elucidated. It belongs to the 5α-steroid reductase family of proteins which are enzymes involved in sterol metabolism in three metabolic pathways: bile synthesis, androgen metabolism and estrogen metabolism. Within the cell, SRD5A3 is localized in the ER and ER-Golgi intermediate compartment.

Protein Domains	Interacting partners
Quaternary structure: No Data Available **Blocks:** 3-oxo-5-alpha-steroid 4-dehydrogenase, C-terminal **InterPro:** Dfg10/SRD5A3 3-oxo-5_a-steroid_4-DH_C **ProtoNet:** Q9H8P0	

SRD5A3-CDG Lay Review

This section is intended for patients, families and caregivers.

Overview

Steroid 5a-reductase type 3 congenital disorder of glycosylation (SRD5A3-CDG, also known as CDG-Iq) is an ultra-rare genetic disorder which results in developmental delays and problems with vision. SRD5A3-CDG disrupts the normal patterns of a critical biological process called glycosylation, in which sugar groups, called ***glycans***, are attached to proteins or fats. Glycosylation is essential for the normal function of proteins and lipids throughout the body and plays an important role in many biological processes. Defects in glycosylation may affect the brain, liver, and heart, as well as the muscle, gastrointestinal, hormone, clotting and immune systems.

The first genetically confirmed case of SRD5A3-CDG was reported in 2010 [1,2]. SRD5A3-CDG is caused by a mutation in the *SRD5A3* gene which provides instructions for making an enzyme called polyprenol reductase. Mutations in the *SRD5A3* gene lead to the production of an enzyme with a lack of or reduced activity. Because SRD5A3-CDG is caused by mutations in a single gene, it is a promising candidate for the development of new therapies ranging from specialized dietary supplementation to advanced medicine like gene therapy. However, because SRD5A3-CDG is so rare, there is currently limited research funding to support the discovery and development of new treatments.

At Cure SRD5A3, our mission is to bring together researchers, families and resources to advance knowledge and find a cure for SRD5A3-CDG. To accelerate scientific breakthroughs, we have assembled a research team of experts from diverse scientific and medical disciplines. Wherever possible, we seek to harness scientific discoveries on SRD5A3-CDG to advance research on other CDGs.

Congenital Disorders of Glycosylation

SRD5A3-CDG belongs to a growing group of inherited metabolic disorders called congenital disorders of glycosylation (CDGs). Since first reported in the medical literature in 1980, over 150 CDG types have been identified [3]. As of 2018, approximately 1,800 CDG cases have been reported worldwide [4] with fewer than 100 cases reported for most types [5]. PMM2-CDG is the most prevalent CDG type with over 1000 cases reported to date [6].

Individual CDGs are caused by a specific mutation in one of the estimated 400 genes that encode proteins involved in glycosylation. A deficiency of one of these proteins can lead to a variety of symptoms affecting multiple organs. Symptoms of CDG typically appear in infancy and early childhood and commonly include developmental delay, failure to thrive, poor muscle tone, neurologic, liver and clotting abnormalities. Affected individuals may also present with skin, eye and heart disease. Symptoms, their severity and disease prognosis, vary greatly depending on the specific CDG type and can even vary among individuals with the same type. The majority of CDGs have no available treatments or cures, but treatments for six CDGs do exist in the form of dietary supplementation.

Currently, CDGs may be broadly classified into five categories depending on the glycosylation pathway(s) affected: (I) disorders of N-linked protein glycosylation, (II) Disorders of O-linked protein glycosylation, (III) disorders of lipid glycosylation, (IV) disorders of multiple glycosylation pathways, and (V) other disorders of glycan metabolism.

CDG types are also classified by the official abbreviation of its defective gene, followed by "-CDG" (e.g. SRD5A3-CDG).

Clinical Features

Clinical Presentation

The clinical presentation of SRD5A3-CDG commonly includes various combinations of [7]:

I. Psychomotor disability – impaired coordination of mental and muscular activity

II. Neurological abnormalities – poor muscle tone (***hypotonia***), lack of muscle control and coordination (***ataxia***), midline brain malformation, underdeveloped cerebellum

III. Abnormalities of facial features [8] – arched eyebrows, wide eyes, shallow nasal bridge, short nose, large mouth

IV. Eye abnormalities – visual loss, involuntary eye movement (**nystagmus**), missing tissue in the iris (**coloboma**), underdeveloped optic disk and nerve

V. Skin symptoms – dark patches (**hyperpigmentation**), dry skin, excessive hair growth, accumulation of dead skin cells (**ichthyosis**), loose skin, thickening of the skin on palms of hands and soles of feet

Symptoms that have been reported in a minority of patients are feeding problems, heart abnormalities, joint hypermobility, and swelling of the liver and spleen.

Symptoms that may develop over time are curving of the spine, cataracts and retinal degeneration (***retinitis pigmentosa***) [7].

Biochemical Abnormalities

Biochemical abnormalities include increased levels of liver enzymes (transaminases) found in the serum, underactive thyroid gland (***hypothyroidism***), and decreased levels of blood clotting factors antithrombin and protein C [7].

Diagnosis

A diagnosis of CDG may be initially suspected based on symptoms, a detailed patient history and thorough clinical exam, however laboratory testing is needed to confirm the diagnosis and identify the specific CDG type. These tests include:

I. Serum carbohydrate deficient transferrin (CDT) analysis

CDT analysis is the initial screening test for patients with suspected CDG. This blood test uses a specialized method call mass spectrometry to analyze the glycan patterns on a protein called ***transferrin***. Transferrin is a glycosylated protein found in the blood and plasma that transports iron throughout the body. This test can only identify some CDG types, including SRD5A3-CDG. Sometimes, transferrin may appear normal in CDT analysis in patients with CDG.

II. Serum transferrin isoelectric focusing (TIEF)

Abnormal transferrin glycan patterns can also be detected through another blood screening test called TIEF. In this test, different forms of transferrin are separated in a gel based upon their electrical charge. Like CDT analysis, this test can only identify some CDG types.

III. Molecular genetic testing

Molecular genetic testing is required to confirm a diagnosis of CDG and to identify the specific type. Genetic testing applies sequencing technology to read patient DNA and identify mutations in the genetic code that cause disease. This has revealed multiple different mutations that cause SRD5A3-CDG.

SRD5A3-CDG cannot be detected by CDT or TIEF analysis in some patients [9], highlighting the importance of genetic testing to obtain a definitive diagnosis.

A representative clinical blood test for CDGs is available from the Mayo Clinic Laboratories under test ID: CDG.

Frequency

To date, 38 genetically confirmed SRD5A3-CDG patients (from 26 families) have been reported in the literature. Most patients have been reported from Afghanistan, the Czech Republic, Iran, Pakistan, Poland, Puerto Rico and Turkey [7].

Inheritance

The majority of CDGs, including SRD5A3-CDG, are inherited in an autosomal recessive fashion with one mutation inherited from each asymptomatic (carrier) parent. Therefore if both parents are carriers, there is a 25% with each pregnancy that the child will have a CDG.

Role of SRD5A3 in Health and Disease

The *SRD5A3* gene encodes an enzyme called polyprenol reductase which is located in a structure within our cells called the endoplasmic reticulum (ER). The ER is where proteins are initially assembled and where the first steps in the glycosylation process take place. Polyprenol reductase (also called SRD5A3) converts a protein called polyprenol into dolichol. The conversion of polyprenol to dolichol is one of many steps in glycosylation and plays a key role in the early steps of this process (Figure 1).

Dolichol is a very important building block in glycosylation. Once dolichol is generated, it is further modified by the addition of phosphates and sugars, becoming a scaffold upon which complex sugar molecules are added onto and then transferred onto proteins. Dolichol is important for several glycosylation pathways in the ER including N-glycosylation, O-mannosylation, C-mannosylation and GPI-anchor biosynthesis [10]. When the production of the dolichol scaffold is disrupted, many proteins are inadequately glycosylated.

SRD5A3-CDG patients, mouse and yeast models with mutations in SRD5A3 have glycosylation defects as well as an accumulation of polyprenol [1,11]. It is not currently known whether incorrect glycosylation, the accumulation of polyprenol, or a combination of both, are responsible for disease in SRD5A3-CDG patients.

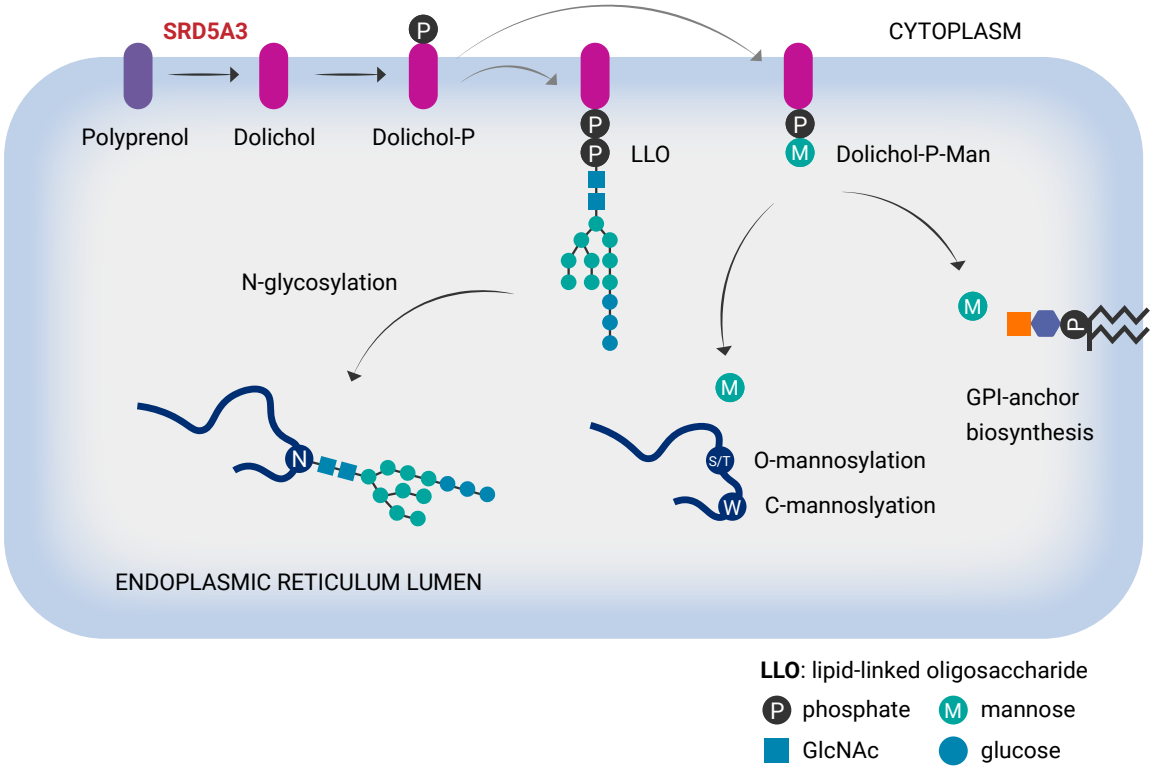


Figure 1: Glycosylation in the Endoplasmic Reticulum

Classification

SRD5A3-CDG is classified as a disorder of multiple glycosylation pathways and more specifically, a disorder of dolichol metabolism.

Treatment

Medical care for CDG patients, including SRD5A3-CDG, typically involves disease management through a combination of physical therapy, occupational therapy such as speech or vision therapy, and/or palliative measures.

Clinical Studies

CDG Natural History Study

There is a lack of information on the natural history of patients with CDGs and no reliable screening tests for many CDG types. The Frontiers in Congenital Disorder of Glycosylation Consortium (FCDGC) is currently conducting a 5-year natural history study on CDGs (NCT04199000). The purpose of this study is to define the natural history and clinical symptoms of CDGs, develop new diagnostic techniques, identify clinical biomarkers that can be used in future clinical trials and evaluate whether dietary treatments improve clinical symptoms and quality of life. Several SRD5A3-CDG patients are currently enrolled in the study which will provide valuable information on many aspects of the disease.

SRD5A3-CDG Patient Characterization Study

Ongoing research at Cure SRD5A3 is focused on characterizing the clinical spectrum of SRD5A3-CDG. The purpose of this study is to learn about different symptoms in SRD5A3-CDG patients and understand how the disease affects their daily lives. This study is an important step towards gathering information that will support the development of therapies and their evaluation in future clinical trials.

Patient Registries

A patient registry is a collection of information from patients about a specific medical condition, as well as its treatment and treatment outcomes. The purposes for patient registries depend upon the specific goals of each registry. This may include but is not limited to: gathering information to help improve quality of life and patient care, learning about disease progression, recruiting patients for clinical trials or answering specific research question(s). Different types of data may be collected in a patient registry including laboratory tests, clinical examination results, patient reported data and biological samples such as blood.

CDG Connect Patient Insights Network (PIN)

CDG Connect PIN is a global patient registry created to advance the understanding of CDGs. CDG Connect PIN collects information with the purpose of understanding the history and progression of CDG, to make it easier for researchers to study CDG, for patients and families to learn about treatment options and for advocates to speak on behalf of the CDG Community. Patients with any CDG type may enroll in the registry.

SRD5A3-CDG Patient Registry

Cure SRD5A3 is currently working towards establishing a global patient registry specifically for patients with SRD5A3-CDG.

Disease Models

The exact nature of CDG biology is not well understood, which makes the development of new treatments a challenge. This is in part due to the rarity of each individual CDG types as well as that there are significant gaps in basic scientific understanding about the effects of incorrect glycosylation on human health. The development of genetically relevant disease models has great potential to accelerate treatment discovery for CDGs.

Cure SRD5A3 researchers are currently developing multiple disease models using patient-derived cells and various organisms to:

- Understand disease mechanisms of SRD5A3-CDG
- Develop a new diagnostic screening test to improve diagnosis
- Identify potential biomarkers

• Identify and evaluate potential therapies

Cell-Based Models

Over the last decade, it has become possible to create patient-specific disease models for the purposes of studying disease mechanisms and testing potential treatments. These ***cell-based disease models*** can be derived from either tissue or blood samples from patients, or non-patient cells that have been engineered to express disease-specific genetic mutations. The key advantage of such patient-derived models is that they contain the same genome as the patient enabling researchers to study the glycosylation defects of individual CDG types. Important patient-specific cell models include:

I. Patient-derived fibroblasts (PDFs) – fibroblast cells derived from skin samples

II. Induced pluripotent stem cells (iPSCs) – fibroblasts or blood cells that have been reprogrammed into a special type master of cell, called a pluripotent stem cell, that can develop into any type of cell in the body

III. iPSC-derived cells – Any cell type, such as a neuron or liver cell, that has been generated by reprogramming iPSCs

Cell-based models have been valuable in investigating underlying disease mechanisms in CDGs, classifying CDG types and assessing the function of proteins encoded by glycosylation genes bearing mutations [12].

Ongoing and future research at Cure SRD5A3 is focused on developing PDF- and iPSC-based models of SRD5A3-CDG. These cell-based models are being used in studies focused on understanding the impact of polyprenol reductase enzyme deficiency, improving disease diagnosis, identifying potential biomarkers, and evaluating potential therapies.

Model Organisms

Model organisms are non-human species that are widely used in the laboratory to research human diseases. Model organisms allow researchers to investigate human diseases when human experimentation would be unethical or unfeasible. Owing to recent advances in genomics, stem cell biology, and ***genome editing***, creating genetically modified organisms as genetic models of human disease has become commonplace. Modern genome editing technologies like CRISPR have enabled efficient generation of disease-relevant model organisms with the same genetic mutations as an individual patient. Model organisms that are commonly used to study disease biology include yeast, worms, flies, fish, mice or rats.

Current research at Cure SRD5A3 includes the development of worms and zebrafish with mutations in the Srd5a3 gene. As Srd5a3-deficient worms mover slower than healthy worms, a drug repurposing screen was carried out to identify potential drugs that improved their movement (unpublished data).

Researchers previously generated knockout mice which did not express the *SRD5A3* gene throughout the whole body. However, these mice like most mouse models of other CDG types, died in utero. To overcome this challenge, researchers have engineered genetically modified mice that have Srd5a3 mutations limited to the cerebellum region of their brain [13]. These mice are viable, show motor and coordination symptoms, and are part of planned studies for new experimental treatments for SRD5A3-CDG.

Biomarkers

Biomarkers, short for biological markers, are measurable indicators of some biological state or condition. They are used for disease diagnosis, the assessment of disease progression and measuring the effects of treatment. Examples of biomarkers include blood pressure, heart rate and specialized genetic tests of blood, urine, cerebrospinal fluid and tissues. Transferrin glycosylation has been the primary biomarker used for CDG screening and therapy monitoring [12]. However, because some CDGs exhibit normal transferrin glycosylation patterns and in several CDGs, transferrin glycosylation patterns normalize with age, the discovery of additional biomarkers is needed [12].

Ongoing research at Cure SRD5A3 is focused on identifying new biomarkers for SRD5A3-CDG to improv diagnosis and therapy evaluation.

Discovering Treatments

As treatment for CDGs is largely supportive, curative therapies are significant unmet need. Several therapeutic approaches are currently under investigation for SRD5A3-CDG, notably drug repurposing and gene therapy.

Dietary Supplementation

To date, six CDGs have benefitted from dietary supplementation, which has helped reduce motor and/or neurological deficits [14]. In each case, adding specific sugars to a patient's diet helped shunt a cellular pathway around a genetic roadblock resulting in treatment. However, more than 100 CDGs remain where effective dietary supplementation has either not yet been identified or attempted, or has not shown a treatment benefit.

Future research at Cure SRD5A3 will be focused on evaluating different dietary supplements like sugars and lipids as treatments for SRD5A3-CDG.

Drug Repurposing

Drug repurposing, also known as ***drug repositioning***, involves re-using an existing drug to treat new diseases. Drug repurposing is often pursued to treat rare diseases as most repurposed drugs have already shown to be safe in humans and can therefore be evaluated in clinical trials more quickly than new drugs. A commonly applied laboratory method for drug repurposing is to perform ***high-throughput screens*** (HTS). HTS involves the use of automated equipment to rapidly test thousands of different drugs and identify potential candidates or 'hits' that can be investigated further. This method can be carried out in human cell-based diseases models, such as patient-derived fibroblasts, or small model organisms such as worms.

In HTS, researchers must have predictive tests, also called ***assays***, that allow them to determine if an experimental treatment improves the health of diseased model being used. Assays can be based on different measurements such as levels of correct protein glycosylation, or changes in animal health, appearance, or behavior. A good assay can clearly detect the difference between healthy or diseased research models, and also whether a treatment is effective in that model when compared to a placebo or a drug that is known to be effective.

Ongoing research at Cure SRD5A3 is focused on carrying high-throughput drug repurposing screens in worm and cell-based models of SRD5A3-CDG.

Gene Therapy

As CDGs are ***monogenic disorders***, meaning that they are caused by mutations in a single gene, they are potential candidates for gene therapy. ***Gene therapy*** is an experimental technique that modifies a person's genes to treat or cure a disease. There are several approaches that may be used in gene therapy:

- Replacing a disease-causing gene with a healthy copy of the gene
- Inactivating a disease-causing gene that is not functioning properly
- Introducing a new or modified gene into the body to help treat a disease

In gene therapy, a carrier called a ***vector***, is required to efficiently deliver genetic material like DNA or RNA to tissues and cells. Gene therapy vectors can be either viral or non-viral. Both forms of gene therapy are currently being studied in clinical trials for genetic diseases.

I. Viral vectors

Viruses are often used as vectors in gene therapy because they have a natural ability to deliver genetic material into cells. These viruses have been genetically modified so that they cannot cause disease when used it people, and are used to carry therapeutic genes into human cells. The most popular gene therapy viral vector is adeno-associated virus, or AAV, which is currently being tested in approximately 200 clinical trials.

II. Non-viral vectors

Non-viral vectors are typically DNA or RNA molecules that have been chemically modified to improve their ability to be absorbed by cells. Non-viral vectors can take deliver therapeutic genes to cells, and their use may be limited to very specific tissues.

Gene therapy is not without its own challenges. CDGs like SRD5A3-CDG affect multiple tissues and almost every cell in the body. Current technology cannot deliver therapeutic genes very widely. This means that regardless of the gene therapy vector used, gene therapy clinical trials in CDGs must focus on tissues that are most significantly affected by disease.

Researchers at Cure SRD5A3 are currently evaluating AAV neurological gene therapy approaches in the cerebellum-specific SRD5A3 knockout mice. These mice are being assessed for improvement in motor coordination defects and the effects of gene therapy on the overall health of the mice are being determined.

SRD5A3-CDG Detailed Review

This section is intended for researchers, healthcare and industry professionals.

Overview

Steroid 5a-reductase type 3 congenital disorder of glycosylation (SRD5A3-CDG, also known as CDG-Iq) is an ultra-rare autosomal recessive genetic disorder. SRD5A3-CDG belongs to a growing group of inherited metabolic disorders called congenital disorders of glycosylation (CDGs). Glycosylation is the process by which sugar residues are attached to proteins or lipids and is essential for a variety of biological processes. Since first reported in the medical literature in 1980, over 150 CDG types have been identified [3]. SRD5A3 encodes polyprenol reductase, an enzyme that catalyzes the conversion of polyprenol to dolichol [11]. Biosynthesis of dolichol is one of the first steps in glycosylation. Dolichol acts as a lipid carrier of a oligosaccharide for N-glycosylation and as carrier for monosaccharides used as donors for N-glycosylation, O-mannosylation, C-mannosylation and GPI-anchor synthesis.

The first genetically confirmed case of SRD5A3-CDG was reported in 2010 [1,2] and to date, 38 cases with molecular confirmed diagnosis have been reported [7]. SRD5A3-CDG is characterized by a highly variable ***phenotype*** typically presenting with psychomotor disability, neurological abnormalities, several visual impairment and ophthalmological abnormalities, facial dysmorphism and skin abnormalities [7]. Symptoms that may develop over time include kyphosis, cataracts and retinitis pigmentosa [7]. There are no approved treatments for SRD5A3-CDG and the majority of CDG types. However, as a monogenic disorder affecting the activity of a well-characterized enzyme, SRD5A3-CDG is a promising candidate for a variety of therapeutic avenues ranging from dietary supplementation to gene therapy.

At Cure SRD5A3, our mission is to bring together researchers, families and resources to advance knowledge and find a cure for SRD5A3-CDG. To accelerate scientific breakthroughs, we have assembled a research team of experts from diverse scientific and medical disciplines. Wherever possible, we seek to harness scientific discoveries on SRD5A3-CDG to advance research on other CDGs.

Clinical Features

CDGs are a clinically heterogenous group of diseases and typically present with multi-systemic manifestations, most commonly developmental delay, hypotonia, failure to thrive, neurological abnormalities, coagulation and liver abnormalities. Affected individuals may also present with eye, skin, and cardiac disease, as well as facial dysmorphisms. Many of these features have been observed in SRD5A3-CDG patients to varying degrees (Table 1) [2,7,11,15].

The clinical presentation of SRD5A3-CDG commonly includes various combinations of [7]:

I. **Psychomotor disability –** impaired coordination of mental and muscular activity

II. **Neurological abnormalities –** hypotonia, ataxia, midline brain malformation, underdevelopment of the cerebellum

III. **Facial dysmorphisms [8] –** arched eyebrows, wide eyes, shallow nasal bridge, short nose, large mouth

IV. **Ophthalmological abnormalities –** nystagmus, visual loss, coloboma, underdevelopment of the optic disk/nerve

V. **Cutaneous symptoms –** hyperpigmentation, dry skin, excessive hair growth, ichthyosis, loose skin, thickening of the skin on the palms of hands and soles

of feet (***palmoplantar keratoderma***)

Symptoms that have been reported in a minority of patients are feeding problems, heart abnormalities, joint hypermobility, and swelling of the liver and spleen. Symptoms that may develop over time are curving of the spine (***kyphosis***), cataracts and retinitis pigmentosa [7]. Recently, hepatic steatosis was reported in a patient with SRD5A3-CDG [16]. Biochemical abnormalities include increased serum transaminases, hypothyroidism, and decreased levels of blood clotting factors antithrombin and protein C [7].

The Sappani Foundation has provided redacted medical records for seven SRD5A3-CDG patients (Table 2).

Table 1. Summary of Phenotypic Features in SRD5A3-CDG Cases Reported in the Literature [15]

		Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Review**	**Review**
Patients	Sex	Female	Male	Male	Female	Female	M:F-5/3	M:F-4/1
Ophthalmic	Colobomas	–	+	–	–	–	6 of 8	1 of 5
	Optic nerve hypoplasia/atrophy	+	+	+	+	+	7 of 8	2 of 2
	Nystagmus	+	+	+	+	+	4 of 8	2 of 2
	Cataracts	–	–	–	–	+	0 of 8	5 of 5
	Retinitis pigmentosa	–	–	–	–	+	0 of 8	2 of 5
Neurologic	Hypotonia	+	+	+	+	+	5 of 8	NR
	Cognitive delays	+	+	+	+	+	8 of 8	4 of 5
	Seizures	+	Febrile	+	–	–	1 of 8	0 of 5
	Ataxia	+	+	–	+	+	2 of 4	2 of 5
	Cerebellar abnormalities	+	+	–	–	–	4 of 8	NR
Dermatologic	Icthyosiform changes	+	+	–	–	–	4 of 8	NR
	Hyperpigmentation-patchy	+	+	–	–	–	2 of 8	NR
	Palmoplantar keratoderma	+	+	–	–	–	3 of 8	NR
	Sacral lesion	+	+	–	–	–	1 of 8	NR
Endocrine	Hypothyroidism	–	+	?	+	–	0 of 7	NR
	Growth hormone def	–	–	?	–	–	1 of 8	NR
Skeletal	Kyphosis	–	–	–	+	–	0 of 8	3 of 5
Lab studies	Increased serum transaminases	–	–	?	?	–	0 of 4	NR
	Type I serum transferrin IEF	+	–	+	?	+	5 of 5	1 of 1
	Decreased anticlotting factors	–	–	?	?	–	2 of 8	0 of 1
	SRD5A3 mutations	c 603G>A (hom)	c 603G>A (hom)	c 57G>A (hom)	c 562 +3delG/ c 921C>G	c 562 +3delG/ c 921C>G		

NR, not reported; ?, not evaluated; hom, homozygous.

[a] Assam et al. [2001], Al-Gazali et al. [2008], Grundahl et al. [2012], Kasapkara et al. [2012], Prietsch et al. [2002].

[b] Kahrizi et al. [2009], Kara et al. [2014].

Table 2. Clinical and Molecular Findings in SRD5A3-CDG

Patient ID	Date of genetic diagnosis	Age	Sex	Descent	SRD5A3 Mutation	Consequence	Clinical Presentation
A*	12/2/2019	16 years	M	–	nonsense mutation c.603 G>A (p. W201X) / c.603 G>A (p. W201X)	protein truncation or nonsense mediated decay; resulting in LOF	Normal head size Normal eye spacing (abnormalities in right pupil) Mild facial abnormalities Thickening of skin at base of sacrum Thickening of skin on palms of hands, soles of feet
B*	–	–	M	Pakistani	nonsense mutation W19X/ W19X	protein truncation or nonsense mediated decay; resulting in LOF	Non-verbal Dependant on gastronomy feeding tube No other organs affected
C	–	–	F	–	c.57 G>A (p.W19X)/ c.57 G>A (p.W19X) heterozygous p.Y440C variant in *ABCA4* (unknown significance)	–	–
D1 †	9/27/2018	14 years	–	–	c.57G>A (p.W19X) / c.57G>A (p.W19X)	–	–
D2 †	9/27/2018	3 years	–	–	c.57G>A (p.W19X) / c.57G>A (p.W19X)	–	–
D3 †	9/27/2018	11 years	–	–	c.57G>A (p.W19X) / c.57G>A (p.W19X)	–	–
E	5/17/2017	4 years	F	–	splice site / missense mutations c.562+3delG (IVS3+3delG)/ c.436 G>A (p.E146K)	mutation in intron 3; suspected disruption of protein function	–
F	7/20/2016	8 years	F	Indian	nonsense mutations c.57G>A (p. W19X)/ c.57G>A (p. W19X)	protein truncation or nonsense mediated decay; resulting in LOF	Transferrin analysis 1; inconclusive Mild ↓ tetrasialotransferrin Mild ↑ disialotransferrin Transferrin analysis 2 & 3; normal Severe ocular phenotype; poor visual acuity, nystagmus, rod-cone dystrophy, retinal nerve fiber layer atrophy Developmental delay Motor and speech delays at 2 months; limited speech, hypotonia

* siblings

† siblings

Diagnosis

Direct molecular genetic testing is the only definitive diagnostic testing method for SRD5A3-CDG diagnosis. However, serum carbohydrate deficient transferrin (CDT) analysis and transferrin isoelectric focusing (TIEF) are the first-line screening tests in patients with suspected CDG, including SRD5A3-CDG [5]. These blood test analyzes the glycan patterns on an abundant serum glycoprotein, called transferrin, by mass spectrometry or isoelectric focusing. Human transferrin has two glycosylation sites, both carrying bi-antennary N-linked glycans with two terminal negatively charged sialic acid residues, and is present in different ***glycoforms***. These glycoforms correspond to the number of sialic acid residues present on the oligosaccharide chains, with tetrasialotransferrin (four sialic acid chains) being the most abundant glycoform in human serum. transferrin CDT and TIEF analyses can only detect CDGs with underlying N-glycosylation defects with sialic acid deficiencies.

Increased abundance of immature transferrin glycoforms (e.g. di- and asialotransferrin) is observed in some patients with SRD5A3-CDG [2]. However in some patients, transferrin glycoform patterns appear normal 9 and glycosylation patterns have been observed to normalize with age in other CDG types [12]. These findings highlight the importance of genetic testing to obtain a definitive diagnosis and the need to develop a more sensitive diagnostic screening test for CDGs.

Challenges in SRD5A3-CDG diagnosis by transferrin analysis are highlighted in testing results obtained in Patient F (Table 2). The initial transferrin analysis revealed an inconclusive sialylation pattern although a CDG diagnosis was expected. Transferrin isoform analysis by HPLC was carried out twice several months later and no abnormal transferrin patterns were detected. These data suggest that transferrin isoform analysis by HPLC cannot reliably detect the glycosylation defect in this SRD5A3-CDG patient.

Classification

SRD5A3-CDG is classified as a disorder of multiple glycosylation pathways and more specifically, a disorder of dolichol metabolism.

Role of SRD5A3 in Health and Disease

Dolichol Biosynthesis

The steroid 5 alpha-reductase type 3 (SRD5A3) gene encodes polyprenol reductase, an enzyme that converts polyprenol to dolichol (Figure 1) [17]. Dolichol is considered the end product of the mevalonate pathway; a metabolic pathway that converts mevalonate into sterol isoprenoids, such as cholesterol, and non-sterol isoprenoids, such as dolichol [10]. The formation of farnesyl diphosphate (FPP) is a critical branchpoint of the mevalonate pathway as it is an intermediate for the biosynthesis of cholesterol, ubiquinone and dolichol. Polyprenol reductase promotes the reduction of the alpha-isoprene unit of polyprenol into dolichol in a NADPH-dependent mechanism [18]. Dolichol is then phosphorylated to become dolichol phosphate (Dol-P) which acts as a lipid carrier for mannose and glucose monosaccharides used as donors for N-glycosylation, O-mannosylation, C-mannosylation and GPI anchor synthesis [18]. Dol-P also serves as a carrier of the oligosaccharide precursor that is transferred en bloc to asparagine residues of nascent polypeptides for N-glycosylation of proteins. This substrate, comprised of Dol-P, pyrophosphate and the oligosaccharide chain, is called the ***lipid-linked oligosaccharide*** (LLO). In addition to the synthesis of Dol-P from dolichol, it is also recycled during N-protein glycosylation.

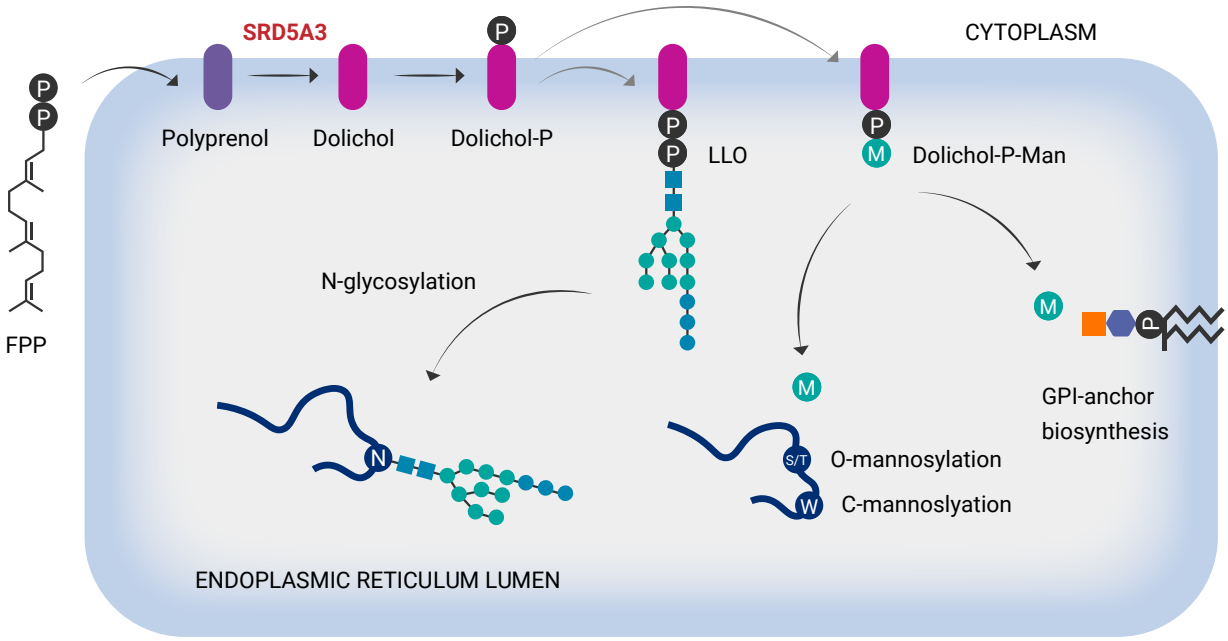


Figure 1. Dolichol synthesis and glycosylation in the ER

Disease Mechanism

As the synthesis of dolichol from polyprenol is one of the first steps in glycosylation, mutations in SRD5A3 result in broad upstream alterations to glycan synthesis and assembly [15]. Insufficient levels of Dol-P and LLO result in defective N-glycosylation and protein ***hypoglycosylation*** [19]. Consistent with the role of polyprenol reductase in N-glycosylation, most SRD5A3-CDG patients have abnormal transferrin profiles [1,2,11,19]. Accumulation of polyprenol has been observed in SRD5A3-CDG patient plasma and fibroblasts, as well as in Srd5a3-/- mice embryos and DFG10 (SRD5A3 ortholog) mutant yeast [1,11,20]. Interestingly, dolichol levels have been observed as normal in patient plasma, urine and fibroblasts, as well as knockout mice, [1,11,20] although hypoglycosylation occurs. These findings suggest the presence of an alternative pathway for de novo dolichol synthesis or a positive feedback mechanism. In support of this hypothesis, whole transcriptome analysis of constitute Srd5a3 knockout mouse embryos revealed upregulation of genes in the mevalonate pathway [1]. It is unknown if such an alternative pathway is producing sufficient levels of dolichol and at the right location to participate in glycosylation.

The degree and mechanism by which hypoglycosylation or polyprenol accumulation drives SRD5A3-CDG pathology is currently unknown. While glycosylation defects in SRD5A3-CDG patients have been found to affect N-glycosylation, dolichol is also required for other glycosylation pathways, therefore it's possible that some of the pathology may derive from disruption of these pathways as well.

Androgen Biosynthesis

SRD5A3 belongs to the 5α-reductase family of enzymes which play a critical role in the biosynthesis of androgens . In addition to its role in dolichol metabolism, SRD5A3 catalyzes the conversion of testosterone (T) into 5-alpha-dihydrotestosterone (DHT). SRD5A3 overexpression is observed in hormone- refractory prostate cancers in which the androgen level is low [21].

Mutational Spectrum

At least 15 variants have been reported in patients with SRD5A3-CDG: 11 nonsense variants, 3 missense variants, and a large deletion [7]. The ***nonsense mutation*** c.57 G>A (p.Trp19X) is a commonly detected allele in patients. This change results in a ***premature termination codon*** and is thus predicted to result in the production of a truncated protein or nonsense-mediated decay.

One patient (Patient C, Table 2) was also reported to be

heterozygous for the Y440C variant (unknown significance) in *ABCA4*. Some *ABCA4* variants are associated with cone rod dystrophy or early-onset macular degeneration [22].

Biomarkers

Transferrin glycosylation has been the primary biomarker used for CDG screening and therapy monitoring, however other biomarkers are used from some CDG types [12]. However, because some CDGs exhibit normal transferrin glycosylation patterns and in several CDGs, transferrin glycosylation patterns normalize with age, the discovery of complementary biomarkers is needed [12]. A recent study evaluated the potential of dolichol in urine and multiple tissues as a potential biomarker for CDGs, including SRDA3-CDG [20]. However, dolichol levels were not significantly different in the urine or fibroblasts of SRD5A3-CDG patients compared to healthy controls [20].

Disease Models

Several SRD5A3-CDG disease models have been generated which have valuable provided insight into the underlying molecular mechanisms in SRD5A3-CDG (Table 3). Ongoing research at Cure SRD5A3 is focused on developing additional models to support drug discovery and therapy development efforts for SRD5A3-CDG.

Yeast (*S. cerevisiae*)

SRD5A3 is the human ortholog of yeast DFG10. Mutant DFG10 yeast display growth delays and hypoglycosylation of the secreted glycoprotein Carboxypeptidase Y. Mutants also display defective metabolism of polyprenol to dolichol, evident by elevated polyprenol and reduced dolichol levels [1].

Table 3. Summary of SRD5A3-CDG models

Model	Mutation	Phenotype	Molecular Mechanisms
S. cerevisiae (Yeast)	Dfg10-100	Defective filamentous growth	Hypoglycosylation of carboxypeptidase Y Elevated polyprenol levels Reduced dolichol levels
C. elegans (Worm)	B0024.13$^{-/-}$	Slow motility Low progeny counts Slow growth/development	ER stress
D. rerio (Zebrafish)	srd5a3$^{-/-}$	No phenotype (assessed for ER stress, larval survival, growth, morphology, and swimming abnormalities)	–
Mouse	Srd5a3$^{Gt/Gt}$	Embryonic lethal beyond E12.5 Embryos were smaller Dilated hearts Open neural tubes	Upregulation of unfolded protein response genes Upregulation of genes in mevalonate pathway Downregulation of genes involved in general cellular metabolic processes and specific embryonic development Elevated polyprenol levels
Mouse (cerebellum specific knockout)	En1-Cre; Srd5a3$^{fl/-}$	Impaired motor coordination Abnormal cerebellum granule cell development	No disrupted ER homeostasis Deregulated abundance of enzymes involved in cholesterol/mevalonate pathway Decreased abundance of N-glycoproteins with high N-glycan multiplicity; particularly IgSF-CAMs
Human (patient-derived fibroblasts)	SRD5A3$^{-/-}$	–	Elevated polyprenol levels Variable levels of lipid-linked oligosaccharide Dolichol levels comparable to healthy controls

Fly (*D. melanogaster*)

A fly *Srd5a3* knockout model has not yet been generated.

Worm (*C. elegans*)

SRD5A3 is the human ortholog of *B0024.13*. Our research partners at Modelis have generated *Srd5a3* deficient worms by CRISPR/CA9 gene editing. *Srd5a3* mutants exhibit a slow motility phenotype (unpublished data) which is the basis for preliminary drug screens in the Cure SRD5A3 research program.

Zebrafish (*D. rerio*)

Our research partners at Modelis have generated *Srd5a3* knockout zebrafish. Mutant zebrafish were assessed for ER stress, larval survival, growth, morphology, and swimming abnormalities but showed no detectable phenotype.

Mice

Several mouse models of Srd5a3 deficiency have been generated.

I. *Srd5a3* knockout mouse

Several groups [1,23,24] generated a homozygous constitutive *Srd5a3* knockout mouse which was embryonic lethal beyond E12.5. Embryos were smaller, displayed open neural tubes and dilated hearts. Whole transcriptome analysis of Srd5a3 mutants revealed activation of the unfolded protein response, upregulation of genes in the mevalonate pathway and downregulation of genes involved in general metabolic processes and embryonic development programs [1]. Polyprenol levels are also elevated in this mouse model of Srd5a3 deficiency [1].

The Jackson Laboratory has recently generated a commercially available conditional-ready floxed *Srd5a3* knockout mouse [24]. This mouse model can be bred to different CRE driver mice to investigate *Srd5a3* deficiency in tissue-specific and inducible models.

II. Cerebellum-specific *Srd5a3* knockout mouse

Recently, a cerebellum-specific *Srd5a3* knockout mouse was generated [13]. The phenotype of this model recapitulates some of the neurological symptoms present in SRD5A3-CDG patients, notably impaired motor coordination. Mice also display abnormal cerebellum granule cell development. Proteomic analysis revealed a decrease in abundance of N-glycoproteins with high N-glycan multiplicity (i.e. have more N-glycosylation sites), specifically proteins belonging to the immunoglobulin super family of cell adhesion molecules (IgSF-CAMs). Several IgSF-CAMs proteins were hypoglycosylated, which resulted in impaired IgSF-CAM-mediated neurite outgrowth and axon guidance in the *Srd5a3* knockout cerebellum. This mouse model revealed the critical role of high N-glycan multiplicity in neuronal adhesion in mammalian brain development.

Patient-Derived Fibroblasts

SRD5A3-CDG patient derived fibroblasts display elevated polyprenol levels, normal dolichol levels and variable levels of lipid-linked oligosaccharides [1,2,11].

Therapies for CDG

There are no treatments available for the vast majority of CDGs. For some, potentially curative therapies currently being used include dietary supplementation and organ transplant. However, for the majority of patients, treatment is mainly supportive and focused on symptom management and improving quality of life. *In vitro* models, such as patient-derived fibroblasts, have been valuable in investigating the underlying molecular mechanisms in CDGs and evaluating potential treatments. Furthermore, the advent of genome-editing techniques has enabled the generation of in vivo models with disease-specific mutations that replicate patient phenotypes. Taken together, these advancements open new avenues for investigating the underlying disease mechanisms in SRD5A3-CDG and the development of therapies for patients.

Dietary Supplementation

An increasing number of studies evaluating the potential of dietary supplementation in CDGs have emerged encouraged by the success of mannose supplementation in MPI-CDG which effectively mitigates disease symptoms [5]. However, in contrast to other glycosylation disorders, MPI-CDG presents without severe neurologi-

cal sequelae and is advantaged by the high bioavailability and cellular uptake of D-mannose [14]. The therapeutic strategy in sugar supplementation is to provide exogeneous monosaccharides to overcome deficiencies in glycosylation by upregulating affected pathways or enabling alternative pathways [3]. The benefits of this approach to treat CDGs include relatively high safety, low cost of compounds and ease of supplementation. To date, exogenous supplementation strategies have been explored for more than 20 CDG types using different disease models with a wide-range of results [14]. Currently, six CDGs benefit from monosaccharide supplementation which include MPI-CDG, CAD-CDG, SLC39A8-CDG ,TMEM165-CDG, PGM1-CDG, SLC35C1-CDG [14].

Unfortunately, dietary monosaccharide supplementation has not proved beneficial for other CDGs types which may be due to a variety of factors such as limited bioavailability, poor blood-brain-barrier penetrance or metabolite toxicity when present at greater than normal levels. Additionally, insufficient stimulation of alternate pathways to circumvent the glycosylation defect may also explain a lack of benefit in dietary supplementation.

Towards a Cure for SRD5A3-CDG

At Cure SRD5A3-CDG, our research program is focused on developing disease altering therapies for SRD5A3-CDG. Towards this goal, we have assembled a team of leading experts in drug discovery, therapeutic development and animal models for rare diseases as well as clinicians with expertise in CDGs. By leveraging the diverse expertise of our team, we aim to accelerate scientific breakthroughs for SRD5A3-CDG and wherever possible, harness these discoveries to advance research on other CDGs.

Ongoing research projects at Cure SRD5A3-CDG include (Figure 2):

- Diagnostic screening assay development and biomarker discovery
- Development and characterization of clinically-relevant cell-based and animal disease models
- Therapy development through drug repurposing, dietary supplementation and gene therapy
- Characterization of the clinical spectrum and natural history of SRD5A3-CDG

Our current research partners include Rarebase, Modelis, the Jackson Laboratory, the Gray lab at UT Southwestern and Dr. Goodspeed at UT Southwestern Children's Medical Center.

Cure SRD5A3 is actively seeking to expand their research program into the development of new disease models, explore additional therapeutic avenues and address critical research questions to support therapy development (Figure 2).

Biomarkers & Development	Disease Models	Clinical Studies	Therapy Development	Research
Diagnostic Test	KO Yeast	Gene Therapy (neurology)	Translational Research*	Patient Registry
Biomarker Discovery	KO Worm	Drug Repurposing		Natural History
	Patient-Derivered Fibroblasts	Small Molecules		
	Cerebellum Specific Conditional KO Mouse	Dietary Supplementation		
	Conditional Ready Floxed KO Mouse	Gene Therapy (ophthalmology)		
	iPSCs	Gene Editing		
	iPSC-Derived Cells	RNA Editing		
	Photoreceptor Specific Conditional KO Mouse	mRNA Therapy		

Figure 2. Roadmap to Cure SRD5A3.

*Coloured boxes indicate of areas of ongoing research and existing models in the Cure SRD5A3 research program. Grey boxes indicate research areas we are in developing and/or are seeking to expand in our research program through partnerships.**

Diagnostic Screening Assay and Biomarker Discovery

As CDT analysis is often inconclusive for SRD5A3-CDG, improved screening methods or the identification of a different biomarker is needed to evaluating clinical response to a therapeutic intervention. Ongoing Research at Cure SRD5A3 is focused on developing a more sensitive CDT test as well as a new MS-based diagnostic screen test using an alternative glycoprotein.

In parallel, an unbiased glycoproteomics approach will also be undertaken to identify potential biomarkers in SRD5A3-CDG blood samples. These screening tests may be used for improved diagnosis and as a clinical endpoint for SRD5A3-CDG, as well as other CDG types for which the transferrin analysis is inconclusive.

Development of Disease Models

The development of patient-specific in vitro models and animal models that replicate patient phenotypes is important for understanding disease mechanisms and evaluating potential therapies for clinical translation. In collaboration with our research partners at Rarebase and the Jackson Laboratory, we are developing and characterizing cell-based and animal research models to support ongoing therapy development for SRD5A3-CDG.

Patient derived fibroblasts, iPSCs and iPSC-derived cells

Global characterization of glycosylation defect and gene expression have not yet been in investigated in SRD5A3-CDG PDFs and other cell types. Additionally, SRD5A3 is downstream of the generation of farnesyl pyrophosphate (FPP) which is a critical branching point in the mevalonate pathway where FPP serves as a substrate for four pathways; protein farnesylation, cholesterol, dolichol and ubiquinone biosynthesis. The levels other metabolites in the mevalonate and branching pathways in SRD5A3-CDG and show a shift in these pathways affects polyprenol and dolichol levels are not known. As PDFs will be used to evaluate a variety of therapeutic candidates, they must be extensively characterized.

Ongoing research is focused on characterizing SRD5A3-CDG PDFs through RNA sequencing, glycoproteomics, metabolomics and lipidomics approaches. It is anticipated that these cells will display defects in glycosylation and polyisoprenoid synthesis that are measurable by MS analysis. These findings will provide screenable phenotypes for carrying out drug screens and evaluating potential therapies *in vitro*.

In addition to PDFs, iPSCs and their differentiated progeny are important cell-based models for studying disease mechanisms, particularly for disorders with a neurological component such as CDGs. Cure SRD5A3 will be developing and characterizing iPSC-derived cell lines from PDFs to better understand disease mechanisms in different cell types and support the evaluation of therapeutic candidates.

Mouse Models

The development of animal models that replication the phenotypes of SRD5A3-CDG patients are important research models for understanding disease mechanisms and are also necessary to assess the safety and efficacy of new therapeutic approaches. Currently, the Gray lab at UT Southwestern is using the cerebellum-specific *Srd5a3* knockout mouse to evaluate gene therapy. In addition to a neurological phenotype, ocular abnormalities and retinal degeneration are observed in SRD5A3-CDG patients. A suitable tissue-specific *Srd5a3* mouse model to study retinal degeneration and evaluate potential vision-restoring therapies for SRD5A3-CDG has not yet been developed.

Our research partners at the Jackson Laboratory have generated a conditional-ready *Srd5a3* floxed mouse strain. Upon breeding to different CRE driver mice, these mouse models will be used to investigate *Srd5a3* deficiency in different tissues or inducible models, as well as the evaluation of potential therapies.

Cure SRD5A3 is seeking research partners to develop and characterize tissue-specific conditional KO mouse models to support the development and evaluation of SRD5A3-CDG therapies targeting the brain or eye.

Therapy Development

As part of the Cure SRD5A3 research program, we are seeking to evaluate multiple therapeutic approaches in parallel using as many disease models as possible. Our research program is currently exploring gene therapy (neurological), drug repurposing and dietary supplementation approaches.

Cure SRD5A3 is seeking research and industry partners with expertise in therapeutic approaches which include small molecule drug discovery, gene therapy, gene editing, RNA editing and mRNA therapy.

1. Drug Repurposing

Drug repurposing continues to be an important route for orphan drug development [25] and has also provided insight into potential therapeutic targets for other CDGs such as PMM2-CDG [26] and NGLY1 deficiency [27]. In partnership with Modelis and Rarebase, we are investigating drug repurposing for SRD5A3-CDG in cell-based and animal models.

Any validated hits from the *C.* elegans and cell-based screens will be evaluated in *Srd5a3* knockout mice to determine if they improve clinically relevant endpoints, such as motor coordination, and assess their safety prior to clinical translation.

I. HTS of Approved Drugs

Modelis has generated *B0024.13* (*SRD5A3* homolog) mutant *C*. elegans lines by CRISPR/Cas9 gene editing which were determined to exhibit a slow motility phenotype. A high-throughput drug repurposing screen of 4,000 approved bioactive molecules has been carried out in the *Srd5a3* deficient worms to identify compounds that ameliorate the motor phenotype. Top candidates are being evaluated in patient-derived fibroblasts by for improvement of glycosylation defects, decreasing polyprenol levels (or other dysregulated metabolites), and increasing dolichol and dolichol-phosphate levels in the ER. It may also be possible to adapt MS-based assays intended for mammalian cells and tissues for worm tissues to directly measure the impact of *Srd5a3* mutations on N-glycosylation or polyisoprenoid biosynthesis.

II. Statins

Genes involved in the mevalonate pathway have been found to be upregulated in *Srd5a3* knockout mice embryos [1]. Select drugs are capable of modulating glycan and lipid biosynthesis and may offer therapeutic avenues to explore for SRD5A3-CDG therapies. These include statins, which inhibit cholesterol and dolichol biosynthesis by competitive inhibition of HMG-CoA reductase. Statins may also reduce the accumulation of polyisoprenoids, although it is not yet known if polyprenol accumulation is drives SRD5A3-CDG pathology. Upon characterization of PDFs, we will be investigating whether statins decrease levels of polyprenol in cells and whether this has an impact improving phenotypes in SRD5A3 KO model organisms.

2. Small Molecule Drug Discovery

In additional to exploring drug repurposing for SRD5A3-CDG, we will carry out HTS screening of diverse small molecule and translational readthrough-inducing drug libraries as well as testing known pathway-specific inhibitors.

I. HTS of Translational Readthrough Compounds

Translational readthrough-inducing drugs (TRIDs) are small molecules that allow translation machinery to bypass a premature-termination codon, resulting in the synthesis of a full-length, potentially functional protein [28]. Given the potential for TRIDs to treat SRD5A3-CDG arising from multiple genotypes, we are interested in screening PDFs with a nonsense mutation in SRD5A3 against a library of translational readthrough compounds. In order to evaluate potential candidates, a suitable readout or assay must first be developed.

Cure SRD5A3 is seeking research collaborators to investigate and characterize translational readthrough compounds for SRD5A3-CDG.

II. Squalene Synthase-Inhibitors

Squalene synthase inhibitors inhibit cholesterol biosynthesis further down the pathway than statins once cholesterol synthesis has been separated from doli-

chol synthesis. The squalene synthase inhibitor zaragozic acid has been shown to improve dolichol-linked oligosaccharide biosynthesis is fibroblasts derived from patients with a dolichol-phosphate-mannose synthase deficiency [29]. However, it is unknown if directing metabolic synthesis towards the dolichol pathway will lead to further accumulation of polyprenol and worsening of symptoms. Characterization of the mevalonate, squalene and dolichol pathways in SRD5A3-CDG primary fibroblasts are critical before evaluating the therapeutic potential of squalene synthase inhibitors.

3. Dietary Supplementation

As dietary supplementation has been a successful treatment strategy for some CDG, Cure SRD5A3 is exploring its potential as a treatment for SRD5A3-CDG.

Cure SRD5A3 is seeking research collaborators to investigate dietary supplementation approaches for SRD5A3-CDG.

I. Sugars and Terpenes

Monosaccharide nutritional therapy alleviates symptoms for several CDG. Terpenes, also called isoprenoids, are large class of natural compounds produced by a variety of plants and have been investigated as treatments in variety of therapeutic areas. Exogenous isoprenoids also regulate HMG-CoA reductase in the mevalonate pathway and may shift towards decreased polyprenol accumulation.

II. Cholesterol

Cholesterol, FPP and exogenous isoprenoids supress HMG-CoA reductase, the rate controlling enzyme of the mevalonate pathway. SLOS is a genetic disorder caused by a mutation in the sterol-7-reductase gene where high urinary excretion of dolichol and ubiquinone is observed in children with this condition. A high cholesterol diet was found to significantly decrease the urinary excretion rate of dolichol, potentially due to a negative feedback mechanism [30]. A high-cholesterol diet may shift the metabolic pathway towards reduced polyprenol accumulation.

4. Gene Therapy

As a rare autosomal recessive monogenic disorder with a clearly identified genetic target, SRD5A3-CDG is a candidate for gene therapy. The expression level of SRD5A3 and tissues that need to be targeted to mitigate pathology in SRD5A3-CDG is currently unknown, although based on known clinical manifestations, the brain and eye are likely critical organs for gene therapy delivery. In addition, it is unclear to what degree the clinical manifestation of SRD5A3-CDG is fundamentally irreversible due to the absence of *SRD5A3* during embryo development. Nonetheless, gene therapy represents a promising experimental strategy for the treatment of SRD5A3-CDG with a focus on neurological and ophthalmological pathology.

I. Gene Therapy (Neurology)

AAV9-*SRD5A3* gene is currently being evaluated in cerebellum-specific *Srd5a3* knockout mice by our research partners in the Grey lab at UT Southwestern. Treated mice are being evaluated for improvement on motor coordination defects by various motor tests. If an effect is observed in the mice, additional safety studies will be performed to enable clinical trials of AAV9-*SRD5A3* in SRD5A3-CDG patients.

II. Gene Therapy (Ophthalmology)

The majority of patients SRD5A3-CDG develop abnormal ocular phenotypes and typically experience early onset vision loss [31]. Early onset-retinal dystrophy has been reported in several patients with homozygous p.W19X mutations in *SRD5A3* and may progress to retinitis pigmentosa over time [31]. Dysfunction of both rod and cone photoreceptors has been reported in these patients. Prior to investigating the potential of gene therapy to treat retinal degeneration in SRD5A3-CDG patients, a tissue-specific conditional *Srd5a3* KO mouse model must first be developed and characterized.

Cure SRD5A3 is interested in i) developing and characterizing a photoreceptor-specific Srd5a3 KO mouse model and ii) will be seeking research or industry collaborators to develop a gene therapy for the eye in SRD5A3-CDG.

Gene Editing

CRISPR-Cas gene editing has the potential to correct the genetic defects in different tissues in SRD5A3-CDG. Precision CRISPR gene editing systems such as base and prime editing, are capable of editing single nucleotides without inducing double-stranded breaks and offer lower off-target activity than Cas9. As many of the mutations identified in SRD5A3-CDG are point mutations, this approach may be useful for across several genotypes.

Cure SRD5A3 is interested in exploring the feasibility of CRISPR precision gene editing approaches as a therapy for SRD5A3-CDG and are seeking research or industry partners.

RNA Editing

Unlike gene editing, ***RNA editing*** systems alter gene expression at the mRNA level through the use of adenosine deaminases acting on RNA (ADARs) [32,33]. ADAR enzymes are capable of effectively editing single nucleotides (A to I; C to U) in diverse cell types and may have reduced off-target effects and immunogenicity [32,33], suggesting a possible therapeutic avenue for treating SRD5A3-CDG of specific genotypes.

Cure SRD5A3 is interested in exploring the feasibility of RNA editing as a therapy for SRD5A3-CDG and are seeking research or industry partners.

mRNA Therapy

mRNA therapies deliver genetic information to the liver where there are translated, thereby generating the encoded protein [34]. mRNA therapies can be used to produce various types of proteins either remain in hepatocytes or are secreted for systemic circulated to other target organs [34]. As most SRD5A3-CDG patients lack polyprenol reductase due to null mutations, mRNA therapy may replace this protein and alleviate symptoms.

Cure SRD5A3 is interested in exploring the feasibility of mRNA therapy for SRD5A3-CDG and are seeking research or industry partners.

Translational Research

Cure SRD5A3 is also seeking research collaborators to engage in a variety of translational research projects to gain a better understanding of disease mechanisms in SRD5A3-CDG and enable breakthrough advancements that are needed to develop therapies as fast as possible.

Specific research questions we are interested in answering include:

- **How is dolichol synthesized in SRD5A3-CDG?**

As dolichol is still present in SRD5A3 knockout cells and organisms, it is possible that there is an alternative pathway for *de novo* dolichol synthesis. Uncovering the genes involved in this pathway will provide insight into potential therapeutic targets for increasing dolichol synthesis. It is also important to know whether this pathway produces ER- localized dolichol that can be phosphorylated to create dolichol-phosphate that participates in glycosylation.

- **How is dolichol synthesis regulated?**

Little is known about how the dolichol pathway is regulated and the proteins that are involved in this network. It is possible that the accumulation of certain metabolites in the upstream, downstream or branching pathways inhibit the activity of other glycosylation enzymes that interact with dolichol. Understanding how dolichol synthesis is regulated may provide insight into opportunities to shift the metabolic flux towards dolichol synthesis or inhibit toxic products to treat SRD5A3-CDG.

- **How does a loss of polyprenol reductase activity contribute to retinal degeneration?**

Retinal dystrophy is observed in several SRD5A3-CDG patients but the disease mechanism is unknown. Developing and characterizing a suitable animal model to study this research question will help better understand the disease and enable the development and evaluation of potential ocular therapies.

- **How does SRD5A3-CDG disease pathogenesis manifest in different cell and tissue types?**

Like most CDG, SRD5A3-CDG is a multisystemic disorder. Although neurological and ophthalmological pheno-

types are observed, the underlying mechanism in these tissues and others is not well understood. Characterizing how a loss of polyprenol activity contributes to disease pathogenesis in different cell and tissue types, such as iPSC derived cells will help better understand the disease and enable drug screening or testing in different types.

Clinical Studies

There is a lack of information on the natural history of patients with CDG, including SRD5A3-CDG. The development of reliable assessments and outcome measures by clinical researchers conducting natural history studies will be instrumental in understanding the efficacy of therapies for SRD5A3-CDG and other CDG types.

Dr. Goodspeed, our clinical research partner at UT Southwestern Children's Medical Center, has conducted a single-year study to characterize the clinical spectrum of SRD5A3-CDG. Additionally, The Frontiers in Congenital Disorder of Glycosylation Disorders Consortium (FCDGC) is conducting a 5-year natural history study on CDGs (NCT04199000). The purpose of this study is to define the natural history and clinical symptoms of CDGs, develop new diagnostic techniques, identify clinical biomarkers that can be used in future clinical trials and evaluate whether dietary treatments improve clinical symptoms and quality of life. Several SRD5A3-CDG patients are currently enrolled in the natural history study. The information gathered from these studies will support the development of therapies for SRD5A3-CDG and their evaluation in future clinical trials.

In parallel, once the SRD5A3-CDG patient registry has been established, Cure SRD5A3 will develop surveys and questionnaires to conduct a longitudinal natural history study.

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Glossary

A

Assay: a laboratory analysis used to detect or measure the biological activity or effects of substance, such as a drug

Ataxia: a lack of muscle control or coordination of voluntary movements

B

Biomarker: a biological molecule found in blood, other body fluids, or tissues that can be used to measure the presence or progress of disease or the effects of treatment

C

Carbohydrate deficient transferrin (CDT): a laboratory test that is used to screen for congenital disorders of glycosylation by detecting abnormal glycosylation patterns on the transferrin protein; can detect some types of N-glycosylation disorders

Cell-based disease model: cells that have been obtained from a tissue or blood sample of a patient, or have been engineered to express a disease-specific genetic mutation

C-mannosylation: a type of glycosylation in which a mannose sugar molecule is attached to the carbon atom of specific molecules in a protein

Coloboma: missing pieces of tissue in the structures of that form the eye, such as the iris

D

Drug repurposing: also called drug repositioning; repurposing existing drugs to treat new diseases

F

Facial dysmorphism: abnormal facial features

G

Gene editing: also called genome editing; a technology in which DNA is inserted, deleted, modified or replaced in a living organism

Gene therapy: a technique that modifies a person's genes to treat or cure a disease; e.g. replacing a disease-causing gene with a healthy copy of the gene

Glycan: any sugar or chain of single sugar molecules, that exist freely or are attached to a molecule such as a protein

Glycosylation: the biological process by which sugars are chemically attached to proteins or lipids

Glycoforms: different molecular forms of a protein, resulting from variability in the number or type of an attached glycan

GPI-anchor biosynthesis: the process by which a lipid that anchors many proteins to the surface of a cell is made

H

High-throughput screening (HTS): a laboratory method that uses automated equipment to rapidly test hundreds of thousands of biological compounds; a drug discovery process

Hyperpigmentation: darker patches of skin

Hypoglycosylation: reduced or insufficient glycosylation

Hypothyroidism: a condition where the thyroid gland does not produce sufficient levels of thyroid hormone

Hypotonia: decreased muscle tone

I

Ichthyosis: a skin condition characterized by dry, thickened, scaly skin

Induced pluripotent stem cells (iPSCs): a type of stem cell that can develop into any type of cell in the body

K

Kyphosis: a spinal condition in which the spine in the upper back has an excessive curvature

L

Lipid-linked oligosaccharide: a chain of sugars linked to dolichol that get transferred onto a protein during the early stages of glycosylation

M

Model organism: non-human species that are widely used in the laboratory to research human diseases

Monogenic disorder: a disorder caused by a mutation in a single gene

Monosaccharide: a single sugar molecule

mRNA therapy: a type of therapy that delivers genetic information (messenger RNA; mRNA) to cells, instructing them to produce the deficient protein

N

N-linked glycosylation: a type of glycosylation in which a small chain of sugars (an oligosaccharide) is attached to the nitrogen atom of specific molecules in protein

Nonsense mutation: also called a stop mutation; a type of mutation that causes a protein to terminate or end its translation earlier than expected

Nystagmus: a vision condition in which the eyes make repetitive, involuntary movement

O

Oligosaccharide: a molecule composed of small number of sugars (monosaccharides) that are linked together

O-linked glycosylation: a type of glycosylation in which a sugar molecule is attached to the oxygen atom of specific molecules in a protein

O-mannosylation: a type of O-linked glycosylation in which a mannose sugar molecule is attached to the oxygen atom on specific molecules in a protein

P

Palmoplantar keratoderma: a group of skin conditions characterized by the thickening of the skin on palms of hands and soles of feet

Patient-derived fibroblasts: fibroblast cells derived from a skin sample of a patient

Phenotype: observable characteristics of an organism

Premature termination codon (PTC): also called a premature stop codon; occurs when a mutation in a sequence of DNA causes translation machinery to stop prematurely, often resulting in a short, incomplete and non-functional protein

R

Retinitis pigmentosa: a condition that involves a break-down and loss of cells in the retina that can lead to vision loss

RNA editing: a technology in which the sequence of RNA is modified before it is translated into a protein by inserting, deleting or substituting a single molecule

T

Transferrin: an abundant protein in the blood that is glycosylated; assessing different patterns of glycans on transferrin can be used to diagnose some CDG

Transferrin isoelectric focusing (TIEF): a laboratory test that is used to screen for congenital disorders of glycosylation by detecting different forms of transferrin that have been separated by electrical charge; can detect some types of N-glycosylation disorders

Translational readthrough-inducing drugs (TRIDs): small molecules that allow translation machinery to bypass a pre-mature stop codon, resulting in the production of a full-length, potentially functional protein

V

Vector: a molecule that is used as a vehicle to deliver foreign genetic information into a cell