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1 **SLC10A7, an orphan member of the SLC10 family involved in**  
2 **Congenital Disorders of Glycosylation.**

3

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1 **Abstract**

2 SLC10A7, encoded by the so-called *SLC10A7* gene, is the seventh member of a human sodium/bile acid  
3 cotransporter family, known as the SLC10 family. Despite similarities with the other members of the  
4 SLC10 family, SLC10A7 does not exhibit any transport activity for the typical SLC10 substrates and is  
5 then considered yet as an orphan carrier. Recently, *SLC10A7* mutations have been identified as  
6 responsible for a new Congenital Disorder of Glycosylation (CDG). CDG are a family of rare and  
7 inherited metabolic disorders where glycosylation abnormalities lead to multisystemic defects.  
8 SLC10A7-CDG patients presented skeletal dysplasia with multiple large joints dislocations, short  
9 stature and *amelogenesis imperfecta* likely mediated by glycosaminoglycan (GAG) defects. Although it  
10 has been demonstrated that the transporter and substrate specificities of SLC10A7, if any, differ from  
11 those of the main members of the protein family, SLC10A7 seems to play a role in Ca<sup>2+</sup> regulation and  
12 is involved in proper glycosaminoglycan biosynthesis, especially heparan-sulfate, and N-glycosylation.  
13 This paper will review our current knowledge on the known and predicted structural and functional  
14 properties of this fascinating protein, and its link with the glycosylation process.

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7 on Congenital Disorders of Glycosylation – from cellular mechanisms to cure – GLYCOLAB4CDG”.

8

9 **Conflict of interest**

10 The authors declare no conflict of interest.

11 **Availability of data and material**

12 Not applicable

13

14 **Code availability**

15 Not applicable

16 **Author contributions**

17 ZD and JD wrote the manuscript with support from DL, VCD and FF. Figures have been made by AL and  
18 DL. All authors discussed and revised the manuscript.

19 **Ethics approval**

20 Not applicable

1 **Consent for publication**

2 Not applicable

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## 1        **Introduction**

2        Glycosylation refers to the post-translational modifications of cellular components such as lipids  
3 and/or proteins by attaching or building up glycan moieties. This highly conserved process occurs  
4 mostly in the compartments of the secretory pathway of mammalian cells (endoplasmic *reticulum* (ER)  
5 and Golgi apparatus). Glycosylation confers specific physicochemical properties needed for the cellular  
6 functions and localizations of proteins or lipids. At the protein level, the N-glycosylation starts in the  
7 ER, while other glycosylation subtypes such as O-glycosylation and/or glycosaminoglycan biosynthesis  
8 occur in the Golgi. Golgi then appears as a major cellular organelle for glycan maturation and  
9 elongation responsible for the great diversity of glycan structures found in humans. The crucial  
10 importance of glycosylation in human beings is underlined by the existence of rare and inherited  
11 diseases, so called Congenital Disorders of Glycosylation (CDG), where more than 130 different  
12 subtypes have been discovered so far and are still expanding (Ng and Freeze 2018). CDG patients  
13 present with multi systemic and metabolic disorders. As described in literature, clinical phenotypes of  
14 CDG patients are broad and can significantly differ according to the CDG subtypes, varying from  
15 skeletal manifestations to intellectual disability and seizures. In this family of diseases where genetic  
16 mutations can directly affect key players in glycosylation, such as glycosyltransferases or enzymes  
17 involved in sugar biosynthesis, a new subtype of genes has been identified in CDG over the last decade,  
18 encoding for proteins involved in Golgi homeostasis and particularly ion homeostasis. It is  
19 unambiguously the case with TMEM165-CDG (Foulquier et al. 2012). TMEM165 is indeed involved in  
20 the Golgi homeostasis regulation of  $Mn^{2+}$ , an ion absolutely required for specific Golgi glycosylation  
21 reactions (Foulquier and Legrand 2020). Interestingly, patients exhibiting mutations which lead to an  
22 impairment of ion homeostasis present with a strong bone defect phenotype. For example, TMEM165-  
23 CDG patients present with dwarfism, scoliosis, osteoporosis, and skeletal dysplasia associated with  
24 intellectual defect. In 2018, a new CDG has been discovered in patients with strong bone defects  
25 combining skeletal dysplasia with multiple large joint dislocations and an *amelogenesis imperfecta*, a  
26 defect in enamel deposition leading to abnormal enamel structure and/or quantity (Dubail et al. 2018).  
27 *SLC10A7* mutations have been identified and characterized as responsible for this CDG subtype.  
28 *SLC10A7* encodes for the SLC10A7 protein, an orphan member of the SLC10 family (Solute Carrier  
29 Family 10) that is composed of seven members. Initially identified as the  $Na^+$ /bile acid symporter  
30 family, this family contains only two members that actually function as  $Na^+$  and bile acid  
31 cotransporters: SLC10A1 and SLC10A2 (Döring et al. 2012). The biological functions of the other  
32 members, SLC10A3, 4, 5 and 6, are still unclear. With regard to SLC10A7, the last discovered member  
33 of the SLC10 family, their cellular and biological functions are rather unknown. This review summarizes  
34 our current knowledge on SLC10A7 and its link with CDG and glycosylation.

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### 1. **SLC10A7, an orphan member of the SLC10 family**

4 The human sodium/bile acid cotransporter family, or SLC10 family, encompasses four main protein  
5 members with reasonably-well defined cellular functions and substrates: SLC10A1, SLC10A2, SLC10A4  
6 and SLC10A6, as well as SLC10A3, SCL10A5 and SLC10A7, three orphan members of the family. This  
7 section will briefly report our current knowledge on all these proteins, except for SCL10A7 which will  
8 be extensively described in the following parts of the manuscript.

9 SLC10A1 and SLC10A2, involved in the recycling of bile acids between the liver and the ileum, are by  
10 far the best characterized transporters of the family (Döring et al. 2012). Whereas SCL10A2, also called  
11 ASBT (apical sodium-dependent bile acid transporter), is found at the surface of enterocytes for the  
12 absorption of bile acids from the intestinal lumen, SLC10A1, or NTCP (Na<sup>+</sup>-taurocholate co-transporting  
13 polypeptide), is expressed by hepatocytes for their re-absorption from portal circulation (Hagenbuch  
14 and Meier 1994, 1996; Wong et al. 1995). Both proteins act as Na<sup>+</sup>-dependent co-transporters of bile  
15 acids like taurocholate, cholate or glycocholate, but a transport activity for sulfated steroid hormones  
16 like estrone 3-sulfate was also demonstrated for SLC10A1 (Ho et al. 2004; Craddock et al. 1998).  
17 Interestingly, mutations of *SLC10A2* have been found in Crohn's disease patients, but the link between  
18 ASBT function and Crohn's disease is still unclear (Xiao and Pan 2017). Till now, neither the structure  
19 of SLC10A1 nor that of SLC10A2 has been solved but the discovery, crystallization and characterization  
20 of two homologs of SLC10A2 in *Neisseria meningitidis* (ASBT<sub>NM</sub>) and *Yersinia frederiksenii* (ASBT<sub>Yf</sub>)  
21 allowed a big step forward for predicting the structure and the mechanism of Na<sup>+</sup>/substrate  
22 cotransport of the main SLC10 proteins (Hu et al. 2011; Zhou et al. 2014).

23 Crystal structures of both ASBT<sub>NM</sub> and ASBT<sub>Yf</sub> revealed that the proteins possess 10 transmembrane  
24 domains (TMD), organized in two inverted repeats of five transmembrane helices forming the core and  
25 panel domains. Like their human homologs, the bacterial proteins exhibit transport activity for bile  
26 acids in a Na<sup>+</sup>-dependent manner. Two Na<sup>+</sup>-binding sites are present in the core domain, whose amino  
27 acid ligands are conserved in SCL10A1 and SLC10A2, and taurocholate binding in ASBT<sub>NM</sub> is supposed  
28 to occur in a hydrophobic cavity between the panel and core domains. Na<sup>+</sup> binding could induce a  
29 conformation change between the core and panel domains, thus permitting the transport of  
30 taurocholate (Hu et al. 2011; Wang et al. 2021).

31 Although *SLC10A3* was the first gene of the SLC10 family to be identified and expressed in a wide range  
32 of tissues (Alcalay and Toniolo 1988), it encodes for a protein whose function is still completely

1 unknown. The gene retrospectively found its place in the SLC10 family because of its sequence identity  
2 of about 20% with the other members of the family.

3 SLC10A4 is expressed in a wide range of human tissues, especially in brain, placenta and liver, and is  
4 localized both at the plasma membrane and in the intracellular compartments (Splinter et al. 2006).  
5 Unlike SLC10A1 and 2, SLC10A4 does not allow cellular taurocholate uptake except after protein  
6 cleavage by proteases, including thrombin (Abe et al. 2013). Although the cellular function of SLC10A4  
7 is not yet fully understood, there is much evidence to qualify this protein as a transporter. Indeed,  
8 more recent studies showed that SLC10A4 is localized in synaptic vesicles (Larhammar et al, 2015).  
9 SLC10A4 expression level is correlated with dopamine uptake even if no evidence for direct transport  
10 of dopamine has been demonstrated. SLC10A4 overexpression can lead to an acidification of the  
11 synaptic vesicles, indicating a potential ionic transport activity.

12 SLC10A5 is a protein predominantly expressed in both liver and kidney. Its localization in the Golgi of  
13 HEK293 and U2SO cells has been demonstrated (Bijsmans et al. 2012), but its function and substrate  
14 specificity are still unknown. Indeed, it was found that SLC10A5 exhibits no transport activity for the  
15 known SLC10A1 and SLC10A2 substrates (Fernandes et al. 2007).

16 SLC10A6 is mainly expressed in testis, but also at relatively high levels in female tissues (breast, vagina,  
17 cervix, placenta), liver and pancreas. Functional studies revealed no transport activity for taurocholate  
18 or any other bile acid except sulfo-conjugated ones like tauroolithocholic acid-3-sulfate, but SLC10A6-  
19 mediated transport in a strictly Na<sup>+</sup>-dependent manner of sulfated steroid hormones was  
20 demonstrated (Grosser et al. 2018). Interestingly, it was shown that SLC10A6 functions may contribute  
21 to hepatic inflammation in mouse liver and macrophages (Kosters et al. 2016) and may be involved in  
22 cancer proliferation in a hormone-dependent manner (Karakus et al. 2018). Indeed, the SOAT  
23 (SLC10A6) protein, encoded by *SLC10A6* is expressed in breast adenocarcinoma, and in mammalian  
24 ductal epithelium cells. Hence, since SLC10A6 is likely involved in hormone transport in these cells, it  
25 could then be a key target in therapies against all stages of hormone-dependent breast cancer (Karakus  
26 et al. 2018).

27 A last member of the SLC10 family, SLC10A7, whose gene defects are responsible for CDG (SLC10A7-  
28 CDG), in contrast to any other member of the family, has been identified a few years ago. Although  
29 both SLC10A7 genetics and pathophysiology of SLC10A7-CDG have been reasonably well described, as  
30 reported in the next chapter, our current knowledge on the structure and functions of SLC10A7  
31 explaining its role in the regulation of glycosylation processes is still sparse. This knowledge and related  
32 clues will be then reported in chapter 3.

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## **2. SLC10A7, genetics and physiopathology: the SLC10A7-CDG**

### **a) Gene structure**

In 2005, Zou et al. performed a large-scale sequencing analysis on a human fetal brain cDNA library. Among other things, they discovered a gene of 2.7-kb, then called C4orf13. This gene contains an open reading frame encompassing nucleotides 218–1237, an in-frame codon stop between nucleotides 155 and 157, and a potential polyadenylation signal in sequence 2305-2310 (Zou et al. 2005). The whole sequence is accessible under the GenBank accession number AY346324. Two years later, Godoy et al. (2007) tried to identify novel proteins containing the SBF (Sodium Bile transporter Family - Pfam PF017) domain, which is a major amino acid sequence signature of the SLC10 family. They used NTCP and ASBT amino acids sequences as queries for GenBank Blast analysis, from which two uncharacterized sequences from mouse liver and rat colon were identified and used for RT-PCR and cDNA cloning. The authors thus obtained 1023 bp sequences exhibiting 14% identity to ASBT and NTCP. The exact same approach was then used to identify 1023 and 1032 bp transcripts from human heart and frog small intestine, respectively. The rat, mouse and human deduced protein sequences share an overall sequence identity of 94%, and the frog sequence exhibits more than 85% identity with the mammalian sequences?. Since all those protein sequences contain the typical SBF domain, the human protein was classified as the seventh member of SLC10 family: SLC10A7 (Godoy et al. 2007). The human *SLC10A7* gene is localized on 4q31 chromosome, and comprises 12 coding exons covering 222 kb, as formerly reported by Zou et al. (2005) (Fig. 1A).

Expression pattern of human *SLC10A7* has been established by RT-PCR analysis. Zou et al. (2005) found higher expression of *SLC10A7* in liver and lungs, a moderate expression in kidney, spleen and thymus, and low expression levels in heart prostate and testis, whereas Godoy et al. (2007) reported a wide *SLC10A7* expression in human tissues, with the highest expression in liver and testis. The expression pattern is similar in mice and rat, but in frog, *SLC10A7* expression was only observed in small intestine, spleen, and skeletal muscle (Godoy et al. 2007). Moreover, the expression pattern has been studied by *in situ* hybridization in mouse embryos and tissue extracts (Dubail et al. 2018). The results showed a ubiquitous expression with, except in cartilage giving birth to long bones and growth plates, where higher expression of SLC10A7 has been observed.

### **b) SLC10A7-CDG patient mutations**

1 In 2018, pathogenic variants in *SLC10A7* gene were identified for the first time in humans. Those  
2 mutations were responsible for a skeletal dysplasia with *amelogenesis imperfecta*, also described now  
3 as short stature, *amelogenesis imperfecta* and skeletal dysplasia with scoliosis (SSASKS,  
4 OMIM#618363). Up to now, nine patients with autosomal recessive mutations in *SLC10A7* have been  
5 described (Ashikov et al. 2018; Dubail et al. 2018; Laugel-Haushalter et al. 2019). Among the eight  
6 pathogenic variants identified in *SLC10A7*, six were present at the homozygous state and two were  
7 compound heterozygous mutations (Table 1). More specifically, three were splice site mutations,  
8 located either in the acceptor sites of exon 9 (c.722-16 A>G), exon 10 (c.774-1 G>A), or in the donor  
9 site of exon 9 (c.773+1 G>A), four were missense mutations located in exon 3 (c.221 T>C [p.Leu74Pro]),  
10 exon 4 (c.335 G>A [p.Gly112Asp] and c.388 G>A [p.Gly130Arg]) and exon 11 (c.908 C>T [p.Pro303Leu])  
11 and one was a nonsense mutation in exon 7 (c.514 C>T [p.Gln172\*]) leading to a premature stop  
12 codon. All the identified mutations were predicted to be damaging either by PolyPhen and Sift  
13 algorithms or by transmembrane prediction software (Fig. 1A & Table1). Functional analyses indeed  
14 confirmed the deleterious impact of the mutations on cDNA and/or protein expression. These findings  
15 allowed to conclude that SSASKS was due to loss-of-function mutations in *SLC10A7*. In addition,  
16 Ashikov et al. (2018) described two other patients with similar clinical features and a complete loss of  
17 *SLC10A7* mRNA arguing for potential mutations in a regulatory element of *SLC10A7*.

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### c) Clinical phenotype

20 All patients with mutations in *SLC10A7* presented very similar clinical phenotypes, mainly  
21 characterized by pre- and postnatal short stature (<-3 SD), large joint dislocations and/or joint  
22 hyperlaxity, abnormal vertebrae with hyperlordosis or (kypho)scoliosis, hypoplastic/hypomineralised  
23 *amelogenesis imperfecta* (defective enamel formation), facial features and Pierre-Robin sequence.  
24 Apart from the two patients described by Ashikov et al. (2018), all other *SLC10A7*-deficient patients  
25 presented with advanced carpal (and tarsal) ossification in early age. Moreover, for several patients,  
26 monkey wrench appearance of the proximal femora was observed in the first months of life, which  
27 associated with short stature, large joint dislocation and advance carpal ossification, is typical of  
28 skeletal dysplasia from the group of chondrodysplasias with multiple joint dislocations (group 20 of  
29 the Nosology and Classification of genetic disorders (Mortier et al. 2019). It is noteworthy that no tooth  
30 abnormalities have been described so far for this group of dysplasia and, thus, *amelogenesis*  
31 *imperfecta* can be considered as a new clinical feature indicative of *SLC10A7* mutations. In most cases,  
32 *amelogenesis imperfecta* was associated with other tooth abnormalities (tooth agenesis, smaller teeth  
33 or dental crowding). Most frequently observed facial abnormalities comprised Pierre-Robin sequence  
34 (micrognathia, cleft palate and glossoptosis), micrognathia and flat face. For one patient, decreased  
35 bone mineral mass compatible with osteoporosis was detected. As it is the oldest patient described so

1 far, it cannot be excluded that other patients will develop with time low bone mineral mass. Finally,  
2 additional features observed included heart defect, moderate hearing impairment (mixed or  
3 sensorineural), mildly impaired intellectual development and obesity.

#### 4 5 **d) *Slc10a7*-deficient animal models**

6 Further strengthening the implication of SLC10A7 in the physiopathology of skeletal dysplasia, two  
7 *Slc10a7*-deficient animal models were generated, both developing severe skeletal dysplasia. In the first  
8 model, *Slc10a7* was inactivated in mouse (Dubail et al. 2018). The resulting mice presented a short  
9 stature detectable at birth, larger ossified tissue in tarsal suggesting advanced ossification and  
10 craniofacial abnormalities. The growth retardation was associated with a strong disorganization of the  
11 growth plate of long bones (tissue responsible for bone growth) and a reduction of bone mass density.  
12 Furthermore, *Slc10a7*-deficient mice exhibited defects in tooth enamel consistent with *amelogenesis*  
13 *imperfecta*. All things considered, the *Slc10a7*-deficient mouse phenotype largely recapitulated the  
14 ones observed in *SLC10A7*-deficient human patients. In the second model, *Slc10a7* was inactivated in  
15 zebrafish using morpholinos (Ashikov et al. 2018). As for mouse models, *Slc10a7* morphant zebrafish  
16 presented with abnormal development of several cartilage elements and a strong reduction in bone  
17 mineralization, once again in accordance with defects described in *SLC10A7*-deficient patients.

### 18 19 **3. Current knowledge and clues on the structure and functions of SLC10A7**

#### 20 **a) Transcript variants and isoforms**

21 Several transcript variants of *SLC10A7* gene have been described in literature so far, in mice but  
22 also in humans. Four variants were formerly reported by Godoy et al. (2007) in human tissues.

23 In a more recent study, a set of five transcript variants was reported, with a numbering from v1 to v5  
24 (Karakus et al. 2020).. The two most expressed variants are v2 and v4, variant v2 corresponding to the  
25 full-length transcript of *SLC10A7* gene. Those transcript variants are summarized in Figure 1B. Variant  
26 v4 differs from variant v2 because of an alternative splicing event that leads to the additional  
27 transcription of exon 11'. Because of this alternative splicing, exon 12 is non-coding, leading the  
28 proteins encoded by variant v2 and v4 to have different C-termini: the protein coded by v2 has a C-t  
29 end encoded by exon 12 while the protein coded by v4 has a C-t end from exon 11'. Karakus et al.  
30 (2020) showed that only v2 and v4 are expressed, and that their expression occurs in most human  
31 tissues, although with slightly different specificities. Variant v2 is indeed found predominant in the  
32 urinary bladder, while v4 is more expressed in the salivary gland.

1 Other variants, v1, 3 and 5, could not be detected in this study. Variant v1 is a non-sense mRNA decay  
2 variant, its transcription being off frame due to a premature stop codon and a skip of exons 8 and 9.  
3 Variant v3 is only made of exons 1 to 4, with an additional alternative exon 4'. The fifth variant (v5)  
4 results from the skip of exon 5. The proteins encoded by those variants are accessible on NCBI, under  
5 the accession numbers NP\_001025487 (v1), NP\_001025169 (v2), NP\_115504 (v3), NP\_001287771 (v4)  
6 and NP\_001304745 (v5). They encode for the SLC10A7 protein isoforms a, b, c, d and e, respectively  
7 (Fig. 1B). Since variant v1 comes from a non-sense mRNA decay, its encoded protein (isoform a) has  
8 been suppressed from the database (Karakus et al. 2020).  
9 At last, two other variants (v6 and v7) can be found in GenBank. Their expression and coding isoform  
10 have not been studied. It is important to note that discrepancies may be found throughout the  
11 literature as regards *SLC10A7* gene and variant expressions in human tissues and organs. The G-TEX  
12 portal for *SLC10A7* (<https://gtexportal.org/home/gene/SLC10A7>) shows a lower expression of *SLC10A7*  
13 in brain, blood and skeletal muscle, and a higher expression in transformed lymphocytes, nerves, and  
14 thyroid. Relative expression in bones is not described.

15

#### 16 **b) Protein primary structure and predicted membrane topology**

17 The full-length SLC10A7 protein (isoform b from transcript variant v2) is composed of 340 amino  
18 acids whose sequence is illustrated in Figure 2A. Although a molecular mass of 37.4 kDa may be  
19 calculated for the protein, immunoprecipitation of FLAG-tagged SLC10A7 from HEK293 cells led to the  
20 detection of two specific bands in western-blot, a major band of 27 kDa and a fainter band of 54 kDa.  
21 Since no predictive N-glycosylation sites were found in SLC10A7, it was hypothesized that the second  
22 band could represent protein dimers (Godoy et al. 2007). In support to this, it has indeed been shown  
23 that dimerization, homo and hetero dimerization, is a common characteristic within the SLC10 family  
24 (Noppes et al. 2019). The Sodium Bile Family (SBF) domain found in SLC10A7 (residues 44-205) shares  
25 a 12-16% identity with the other SLC10 family members (Godoy et al. 2007). Bioinformatics studies  
26 formerly predicted a membrane topology model of either 10 or 9 TMDs. In the 10 TMD model, N and  
27 C-termini have been predicted as intracellular, while in the 9 TMD model, the N-t end was predicted  
28 to be intracellular and the C-t end extracellular. Actually, owing to strategical insertion of HA and FLAG  
29 tags in the SLC10A7 protein expressed in HEK293T cells, followed by immunofluorescence staining with  
30 different permeabilization protocols, the 10 TMD model has been validated (Godoy et al. 2007) (Fig.  
31 2). According to these findings, and by referring to the structural homologies of SLC10A7 with bacterial  
32 homologs ASBT<sub>NM</sub> and ASBT<sub>Vf</sub>, the structure of the protein may be predicted, and is presented in  
33 section 3c (Fig. 2).

34

### 1           c) Predicted 3D structure

2           Till now, as mentioned in section 1, none of the proteins of the SLC10 family have seen their  
3 structure experimentally characterized. However, the crystal structure in detergent conditions of a  
4 bacterial SLC10 homolog from *N. meningitidis* (ASBT<sub>NM</sub>) (Hu et al. 2011), followed by the structure in a  
5 lipid environment of its homolog from *Y. frederiksenii* (ASBT<sub>Yf</sub>) (Zhou et al. 2014; Wang et al. 2021), has  
6 been solved. This was particularly useful for predicting the structures of proteins with similar Na<sup>+</sup>-bile  
7 acid cotransport functions, most especially SLC10A1 and SLC10A2, as reported earlier (Döring et al.  
8 2012; Claro da Silva et al. 2013). Despite the fact that SLC10A7 markedly differs from the other SLC10  
9 family members, owing to its low sequence identity, higher gene complexity, main location within the  
10 secretory pathway, and the evidence that it is not a transporter of bile acids. strong structure  
11 homologies may also be found between SCL10A7 and the bacterial ASBT homologs, most particularly  
12 ASBT<sub>Yf</sub>. (Fig. 2). Like ASBT<sub>Yf</sub> and ASBT<sub>NM</sub>, SCL10A7 is predicted to have 10 domains with both N- and C-  
13 t ends towards the cytosolic side (Fig. 2A). Interestingly, the predicted SLC10A7 scaffold is very similar  
14 to that of bacterial ASBT, with a core domain dedicated to the binding of ions, and a panel domain  
15 delimiting a large cavity likely involved for the binding and transport of an organic compound (Fig. 2B  
16 & C, grayed area A). This strongly suggests that SLC10A7 may act, in a similar way to bacterial ASBT,  
17 human SLC10A1 and SLC10A2, as a transporter of organic molecules, which still need to be  
18 characterized. Furthermore, the overall folding of the core domain seems to be homologous to the  
19 Na<sup>+</sup>-bile acid cotransporters, most particularly with the presence of a central cavity containing the two  
20 Na<sup>+</sup>-binding sites, mentioned in section 1, mainly formed at the interface between the non-helical  
21 segments of TM4 and TM9 (residues 113-121 and 277-281) (Fig. 2A and Fig. 2B & C, grayed area B).  
22 However, it may be observed that most residues involved in both Na<sup>+</sup>-binding sites 1 and 2 of ASBT<sub>Yf</sub>  
23 (Zhou et al. 2014; Wang et al. 2021) whose homologous positions on SLC10A7 are indicated in Figure  
24 2A by the yellow- and green-circled amino acids, are poorly conserved. More particularly, two amino  
25 acids of TM9 critical for binding Na<sup>+</sup> in ASBT<sub>Yf</sub>, E<sub>254</sub> and Q<sub>258</sub>, highly conserved in SLC10A1 and SLC10A2,  
26 are found as C<sub>274</sub> and K<sub>278</sub> in SLC10A7. Those two amino acids are extremely conserved in SLC10A7  
27 orthologs (personal data). This strongly suggests that the transport function of SLC10A7, if any, may  
28 not depend on Na<sup>+</sup> binding. Interestingly, it may be observed that all single point mutations found in  
29 SLC10A7-CDG patients and reported in section 2, except for P<sub>301</sub>, are located within the core domain,  
30 at the vicinity of the amino acids potentially involved in ion binding.

31

### 32           d) Protein localization

1 Given the lack of SCL10A7-specific antibodies suitable for immunochemistry, the subcellular  
2 localization of the protein is not completely solved yet. The use of different SLC10A7 tagged forms and  
3 cellular models, led to different observations. First, Godoy et al. (2007) found most of SLC10A7  
4 localized at the plasma membrane in HEK293T cells, with a non negligible part in the ER. While such  
5 expression at the plasma membrane was further supported using the same cell line (Dubail et al. 2018),  
6 and also for Rch1p, the SLC10A7 yeast ortholog (Jiang et al. 2012), the localization of SLC10A7 was  
7 reported along the compartments of the secretory pathway in several studies. In particular, a specific  
8 Golgi distribution of SCL10A7 in both HeLa cells and human fibroblasts was demonstrated by Ashikov  
9 et al. (2018). This however does not seem to be restricted to the Golgi as an ER localization of SLC10A7,  
10 colocalizing with other ER-specific proteins like STIM1, was observed in HAP1 cells (Karakus et al. 2020).

11 Although all these data do not allow to precisely define the subcellular localization of SLC10A7, which  
12 probably differs according to the type and physiological status of cells, they reasonably support its  
13 presence in the different compartments of the secretory pathway, and more especially in the Golgi  
14 where the maturation of glycans and glycosaminoglycans occurs.

15  
16

#### **e) Link with Ca<sup>2+</sup> homeostasis**

17 CaRch1 and ScRch1, two orthologs of SLC10A7 in *Candida albicans* and *Saccharomyces cerevisiae*,  
18 respectively, helped to highlight the function of SLC10A7 in cellular Ca<sup>2+</sup> homeostasis (Jiang et al. 2012;  
19 Zhao et al. 2016). This link was further confirmed through experiments in human cells (Karakus et al.  
20 2020).

21 Indeed, it has been shown that in  $\Delta$ -CaRch1 *C. albicans* cells, the Ca<sup>2+</sup> entry in cells is increased,  
22 together with the cytosolic Ca<sup>2+</sup> levels, leading to a strong activation of the calcineurin pathway. This  
23 pathway is activated in presence of high Ca<sup>2+</sup> concentrations and is used by yeasts to decrease the Ca<sup>2+</sup>  
24 quantity in the cytoplasm: Ca<sup>2+</sup> can activate the calcineurin phosphatase, that will itself  
25 dephosphorylate and activate Crz1, a transcription factor that will increase the transcription of genes  
26 involved in Ca<sup>2+</sup> storage in organelles (like *Pmr1*) or Ca<sup>2+</sup> efflux at the plasma membrane (Jiang et al.  
27 2012). In a study by Zhao et al (Zhao et al. 2016), the authors demonstrated that, shortly after an  
28 important extracellular Ca<sup>2+</sup> pressure, Rch1 is overexpressed *via* the calcineurin pathway and found at  
29 the plasma membrane, especially at the bud in budding yeast and in a few intracellular puncti. It is  
30 assumed that such Rch1 overexpression aims at negatively regulating Ca<sup>2+</sup> homeostasis at the plasma  
31 membrane to fine tune intracellular Ca<sup>2+</sup> homeostasis and provide negative feedback on the  
32 calcineurin pathway. Although the transport activity of Rch1 and its exact cellular functions have not

1 been characterized yet, these data clearly indicate that the yeast SLC10A7 homolog acts, directly or  
2 indirectly, as a negative regulator of cytosolic Ca<sup>2+</sup> (Zhao et al. 2016).

3 In human cells, the cellular Ca<sup>2+</sup> entry mostly occurs *via* the Store Operated Channels (SOC). These  
4 channels that are expressed at the plasma membrane transport extracellular Ca<sup>2+</sup> into the cytoplasm.  
5 ORAI1 is the main SOC in human cells. As illustrated in Figure 3 (adapted from Lu and Fivaz 2016),  
6 depletion of Ca<sup>2+</sup> from the ER store leads to the oligomerization of STIM1 proteins, then acting as a  
7 direct reticular sensor of Ca<sup>2+</sup> concentration. Once oligomerized STIM1 then triggers the activation of  
8 the plasma membrane channels ORAI1, by interacting with ORAI1 at ER/plasma membrane contact  
9 puncti. This leads to a cellular Ca<sup>2+</sup> entry in the cytoplasm *via* these ORAI1 channels. The cytosolic Ca<sup>2+</sup>  
10 is then transported and relocalized from the cytoplasm into the ER *via* the SERCA pumps, thus allowing  
11 repletion of ER Ca<sup>2+</sup> stores (Fig. 3). Very interestingly, Karakus et al. (Karakus et al. 2020) showed that  
12 in SLC10A7 KO HAP1 cells, Ca<sup>2+</sup> entry *via* the SOC channels is increased, as compared to wild-type HAP1  
13 cells, whereas the overexpression of SLC10A7 in those cells inhibits the SOC-dependent Ca<sup>2+</sup> entry.  
14 These results then strongly suggest that SLC10A7 expression is negatively correlated with the SOC-  
15 dependent Ca<sup>2+</sup> entry in cells. In addition, the authors showed that the overexpression of mutated  
16 SLC10A7 patient forms does not rescue the Ca<sup>2+</sup> entry increase in SLC10A7 KO HAP1 cells, thus  
17 indicating that the mutations affect the functionality of SLC10A7. At last, they found that SLC10A7 co-  
18 localizes with STIM1 and SERCA in the ER, and thus hypothesized that SLC10A7 may be a regulator of  
19 one or more of those proteins involved in Ca<sup>2+</sup> homeostasis in human cells (Karakus et al. 2020)  
20 (illustrated in Fig 3).

21 Remarkably, most patients having genetic mutations affecting STIM1 or ORAI1 functionality present  
22 with an *amelogenesis imperfecta* and hypocalcification of enamel, two specific clinical phenotypes  
23 associated to SLC10A7-CDG patients (Lacruz and Feske 2015). This strongly supports the hypothesis of  
24 a link between SLC10A7 and the STIM1/ORAI1 pathway in regulating cellular Ca<sup>2+</sup> homeostasis and this  
25 observed specific phenotype.

26 Since Ca<sup>2+</sup> has pleiotropic cellular roles, it may be expected that any deficiency of SLC10A7 might  
27 negatively impact many cellular mechanisms, including of course the glycosylation processes, as  
28 reported thereafter, but also any other intracellular signaling and trafficking process.

29

#### 30 **f) Impact of SLC10A7 deficiency on glycosylation**

31 The importance of SLC10A7 for glycosylation has emerged with the discovery by Ashikov et al.  
32 (2018) of *SLC10A7* mutations in CDG patients exhibiting glycosylation defects but no mutations in  
33 already-identified CDG-linked genes. This allowed the identification of *SLC10A7* gene deficiencies as a

1 cause of a new CDG and the observation of a characteristic glycosylation signature for this specific  
2 CDG. In particular, some abnormalities in N-glycan biosynthesis, especially occurring in the Golgi part  
3 of the N-glycan maturation process, were depicted by glycomic analysis of patient plasma  
4 glycoproteins. N-glycosylation is initiated in the ER by the en bloc transfer of the oligosaccharide  
5 precursor onto proteins. This precursor is composed of two types of carbohydrates: N-  
6 acetylglucosamine and mannoses. Then, the proteins are sent to the Golgi apparatus where their N-  
7 glycans are matured, to include N-acetylglucosamine, mannose, galactose and terminal sialic acid  
8 residues. However, in SLC10A7-CDG patients, high-mannose N-glycans, only composed of N-  
9 acetylglucosamine and mannose residues, are overrepresented, and the sialylation degree of complex  
10 (mature) N-glycans is decreased. Those N-glycans abnormalities are rather difficult to understand but  
11 indicate that SLC10A7 deficiency disturbs many steps of the N-glycan maturation through the different  
12 cisterna of the Golgi apparatus (Ashikov et al. 2018). In addition to the observed N-glycan defects, an  
13 impact on the biosynthesis of glycosaminoglycans have been reported in both SLC10A7 patients'  
14 fibroblasts and SLC10A7 KO mice (Dubail et al. 2018). Although the total GAG contents of patient  
15 fibroblasts and cartilage extracts of SLC10A7 KO mice were unchanged as compared to controls, the  
16 amount of heparan-sulfate (HS) was surprisingly significantly decreased, with about 2- and 2.5-fold less  
17 HS found in SLC10A7-deficient fibroblasts and SLC10A7 KO mice cartilage extracts, respectively. Since  
18 those HS chains were sulfated and had a length similar to HS chains of control cells, it was concluded  
19 that the defect in HS chains was more quantitative than qualitative. The amounts of chondroitin-  
20 sulfate (CS) chains were also assessed by immunostaining in growth plates of control and SLC10A7 KO  
21 mice. Unlike HS, no decreased amounts of CS could be observed, but a higher density of CS at close  
22 proximity of chondrocytes in KO mice than in control mice was found (Dubail et al. 2018).

23 As depicted in Figure 4, which illustrates the impacts of SLC10A7 deficiency on both N-glycosylation  
24 and GAG biosynthesis pathways, GAG biosynthesis starts in the Golgi by the synthesis of a linker,  
25 common to all GAG subtypes. The difference between CS and HS deals with the transfer to the linker  
26 of a GalNAc by the CSGalNACT1/2 or a GlcNAc by the EXTL1-3, respectively (Chen et al. 2018). Since  
27 SLC10A7 deficiency decreases the amounts for HS, not CS, it may be assumed that the GAG linker  
28 biosynthesis is not affected by the deficiency. In contrast, although this cannot still be explained, the  
29 glycosyltransferases involved in HS biosynthesis, but not CS, seem to be impacted by SLC10A7  
30 deficiency. Interestingly, other genetic diseases affecting the biosynthesis of GAGs, such as EXTL1-3-  
31 CDG, caused by a defect of the N-acetylglucosaminyltransferase I involved in the initiation of  
32 elongation of HS chains, also exhibit a clinical phenotype including a bone mineralization defect or  
33 skeletal dysplasia (Volpi et al. 2017).

1 Of course, it cannot be excluded that the  $\text{Ca}^{2+}$  homeostasis impairment, caused by SLC10A7 deficiency,  
2 leads to the abnormal glycosylation phenotype. Indeed, the observed intracellular  $\text{Ca}^{2+}$  increase could  
3 affect the transcription level, vesicular trafficking speed and activity of any protein involved in Golgi  
4 glycosylation process. In particular, it is worthy to remind that the availability of both  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  in  
5 the organelles is an important factor for Golgi glycosylation. Actually, the first Golgi mannosidases  
6 involved in the trimming of high mannose N-glycans are  $\text{Ca}^{2+}$  dependent while most of the Golgi  
7 glycosyltransferases require  $\text{Mn}^{2+}$  as cofactor. Hence, it is likely that any imbalance of ion homeostasis  
8 would lead to specific Golgi glycosylation defects.

9 To summarize, SLC10A7 deficiency impairs N-glycan maturation and sialylation, HS expression and CS  
10 localization thus affecting a large diversity of glycosylation processes in the Golgi apparatus, from the  
11 ERGIC to the trans-Golgi network (Fig. 3).

12

### 13 **Conclusion**

14 Although the exact intracellular localization of SLC10A7 still needs to be outlined, the strong  
15 impact of its deficiency on glycosylation, all along the secretory pathway, together with the link with  
16  $\text{Ca}^{2+}$  homeostasis, highlights this putative transporter as a key player in ER/ Golgi ion homeostasis as  
17 well as glycosylation. Unravelling the role of SLC10A7 in Golgi ion homeostasis could then lead to the  
18 discovery of simple new therapeutic pathways for SLC10A7-CDG patients, as it was the case for  
19 TMEM165-CDG (Houdou et al. 2019). Ion transport and regulation in the Golgi apparatus are still poorly  
20 understood, and their crucial roles in Glycosylation processes has clearly been underestimated. Future  
21 research are needed to disentangle the function of SLC10A7 in Golgi ion homeostasis regulation and  
22 Golgi glycosylation process.

23

24

### 25 **Legends to Table and Figures**

26

27 **Table 1 : Mutations in SLC10A7-CDG patients** (inspired from Karakus et al. 2020)

28

### 29 **Figure 1: SLC10A7 genomic organization and transcript variant representation**

30 **A** Schematic representation of SLC10A7 genomic coding sequence. It is divided into 12 exons (white-  
31 numbered blue boxes). The length of each exon is proportional to their base content. The introns are  
32 not scaled. The numbering under each exon is based on coding nucleotides (Zou et al. 2005). cDNA  
33 Patients mutations are spotted with red arrows and noted in red. **B** SLC10A7 most common variants,

1 v2 and v4. Exon 11' is only present in v4 whose exon 12 represented with a dotted line is non coding.  
2 Each variant can be found by an Ensembl transcript number annotated under the sequence.

3  
4

5 **Figure 2: Predicted topology and 3D structure of SLC10A7**

6 A search on the PHYRE2 server (<http://www.sbg.bio.ic.ac.uk/~phyre2/>) using the human SLC10A7  
7 isoform b protein chain (UniProtKB/Swissprot ID Q0GE19-2) indicates a 100% probability/confidence  
8 that amino acid residues 6-332 of SLC10A7 match with the apical sodium-dependent bile acid  
9 transporter (ASBT; also known as SLC10A2) homologue from *Yersinia frederiksenii* (ASBT<sub>Yf</sub>) whose  
10 structure in a lipid environment was solved at 1.95 Å resolution (PDB entry 4N7W, Zhou et al. 2014).  
11 This ultimate homology probability, together with a 21% sequence identity between SLC10A7 and  
12 ASBT<sub>Yf</sub>, give reasonably accurate the topology model depicted in the scheme in panel A and the overall  
13 SLC10A7 fold shown in panels B and C. (A) Putative schematic 2D topology of the human SLC10A7  
14 isoform b protein chain showing the 10 transmembrane domains (TM1-TM10) predicted by both  
15 TMHMM v.2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>) and PHYRE2 servers. Both N- (N-t) and C-  
16 terminal (C-t) ends are located at the cytosolic side. The transmembrane segments belonging to the  
17 predicted functional domains of SLC10A7 described in the text and illustrated in B and C, namely the  
18 core domain (TM3-5, TM8-10) and the panel domain (TM1-2, TM6-7), are indicated. The luminal side  
19 and cytoplasmic loops are indicated as L1-L5 and C1-C4, respectively. The one-letter-code amino acids  
20 in the black- grey- and white-filled circles correspond to the identical, highly similar and non-conserved  
21 residues between SLC10A7 and ASBT<sub>Yf</sub>, respectively, as determined by primary sequence alignments  
22 in Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) (Madeira et al. 2019). The red-circled  
23 amino acids are those found mutated in SLC10A7-CDG patients (see section 3 of the manuscript), with  
24 red arrows indicating the amino acid changes. The yellow- and green-circled amino acids are located  
25 at positions corresponding to the residues identified in the Na<sup>+</sup>-binding sites 1 and 2 of ASBT<sub>Yf</sub>,  
26 respectively (Zhou et al. 2014; Wang et al. 2021). (B and C) Model of SLC10A7 predicted on PHYRE2  
27 using the structure of ASBT<sub>Yf</sub> (PDB entry 4N7W) as a template. Rainbow-color fold representation was  
28 obtained using the UCSF ChimeraX software (Goddard et al. 2018). The numbering of TM domains and  
29 loops is the same as in panel A. The side view (B) and the top view (C) of the predicted SLC10A7 model  
30 are shown. The core and panel domains are indicated. The grayed areas roughly indicate the locations  
31 of the bile acid pocket (area A) and the two Na<sup>+</sup>-binding sites (area B) characterized in ASBT<sub>Yf</sub> (Zhou et  
32 al. 2014; Wang et al. 2021).

33

34 **Figure 3: Negative regulation by SLC10A7 of SOC-dependent cellular Ca<sup>2+</sup> entry in response to ER Ca<sup>2+</sup>**  
35 **depletion.**

1 This scheme, inspired from Lu and Fivaz (2016), shows the principal molecular components (ORAI1 and  
2 STIM1) and their interactions permitting the store operated  $\text{Ca}^{2+}$  entry (SOCE). When ER  $\text{Ca}^{2+}$  stores  
3 are full (left side), STIM1 is dispersed throughout the ER. In conditions of ER  $\text{Ca}^{2+}$  depletion, STIM1  
4 oligomerizes (red arrows), recruits and interacts with ORAI1 at ER-PM contact sites, then allowing  
5 cellular  $\text{Ca}^{2+}$  entry (blue arrows) and replenishing of stores *via* the SERCA pumps. SLC10A7, mainly  
6 expressed in the secretory pathway, ER and/or Golgi, seems to act as a negative regulator of SOC-  
7 dependent  $\text{Ca}^{2+}$  ER replenishing by possibly interacting with one or more components of this pathway  
8 (ORAI1, STIM1 and/or SERCA) (black arrows). The solute transport activity of SLC10A7, still not  
9 characterized (green arrows), may also account for the negative regulation of  $\text{Ca}^{2+}$  entry and storage.  
10 Figure was built using Servier Medical Art graphics (smart.servier.com).

11

12 **Figure 4: Schematic representation of the impact of SLC10A7 on the N-glycosylation process and**  
13 **heparan sulfate biosynthesis**

14 SLC10A7 deficiency impacts glycosylation processes in the different cellular compartments and Golgi  
15 cisterna represented. N-glycosylation and heparan sulfate (HS) synthesis (red frames) are affected.  
16 Regarding N-glycans maturation, high mannose structures such as  $\text{Man}_9\text{GlcNAc}_2$  increase. A decrease  
17 in the sialylation degree of complex N-glycans is also found. Heparan Sulfate biosynthesis is affected  
18 in SLC10A7 deficiency. A general decrease is observed but without affecting the quality of the HS  
19 structures. The substitution of the glycans is not fully represented; only the remaining structure at the  
20 entrance of each cisterna is shown. Phosphorylation is represented by the letter « P ». The symbol  
21 nomenclature for glycan structure is depicted in the bottom left-hand corner.

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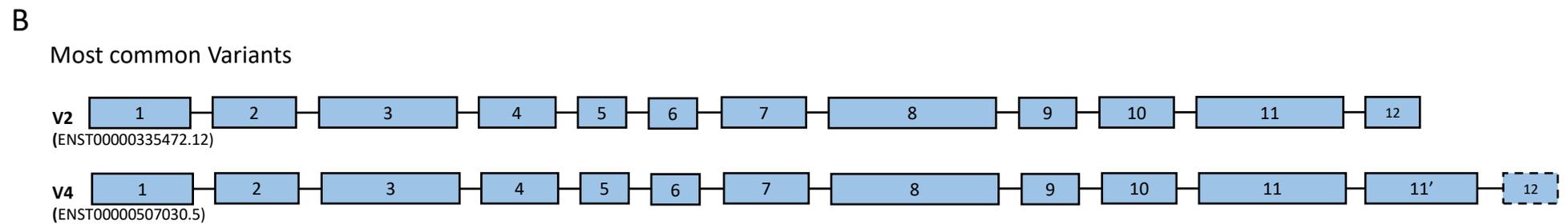
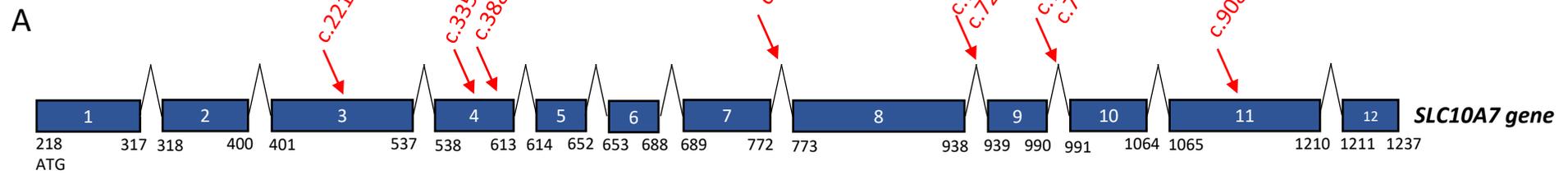
17  
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**Table 1**

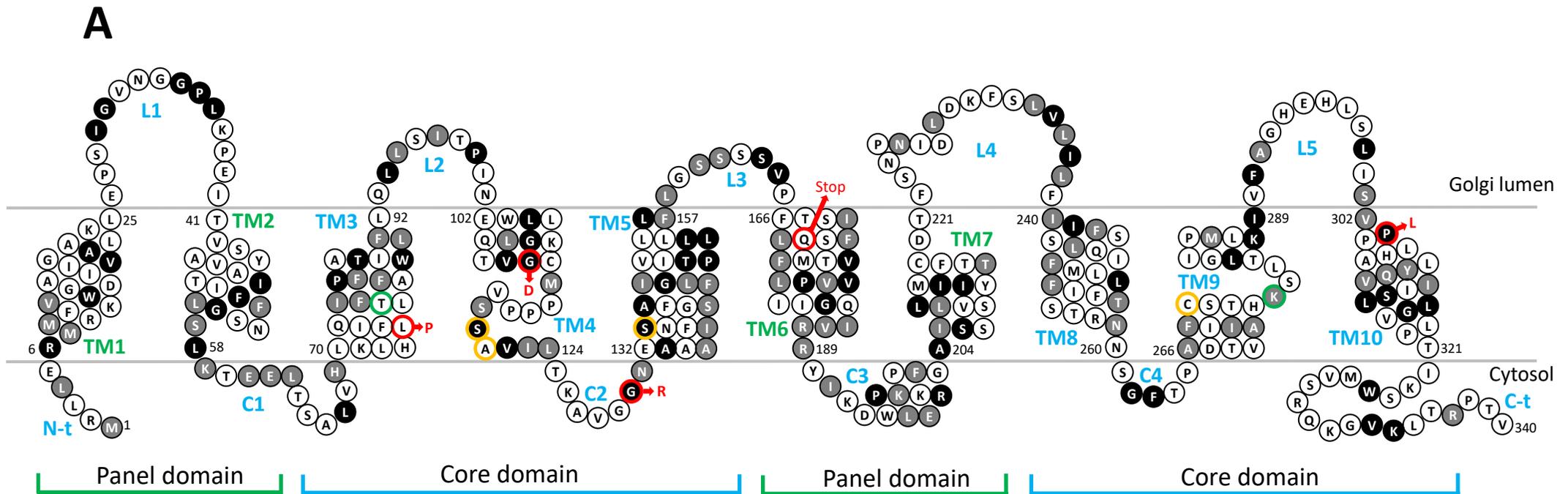
<b>Nucleotide change</b>	<b>Amino acid change</b>	<b>Location</b>	<b>Status</b>	<b>Comment</b>
c.221T>C	Leu74Pro	Exon 3	Homozygous	Mutation affects a highly conserve amino acid in the third predicted transmembrane helix
c.335G>A	Gly112Asp	Exon 4	Compound Heterozygous with c.722-16A<G	Mutation leads to the disruption of the fourth transmembrane domain
c.388G>A	Gly130Arg	Exon 4	Homozygous	Missense mutation which affects a highly conserve amino acid
c.514C>T	Gln172*	Exon 7	Homozygous	Mutation leads to a premature codon stop
c.722-16A>G	Gly112ASP	Intron 8	Compound Heterozygous with c.335G>A	Premature codon stop. Exon 9 skipped
c.773+1G>A	?	Intron 9	Homozygous	Splice mutations in intron 9 leading to exon 9 skipping.
c.774-1G>A	?	Intron 9	Homozygous	Splice mutations in intron 9 leading to exon 10 skipping
c.908C>T	Pro303Leu	Exon 11	Homozygous	Mutation in the tenth transmembrane domain
-	-	-	-	Absence of SLC10A7 cDNA and complete loss of SLC10A7 protein

## **Abbreviations**

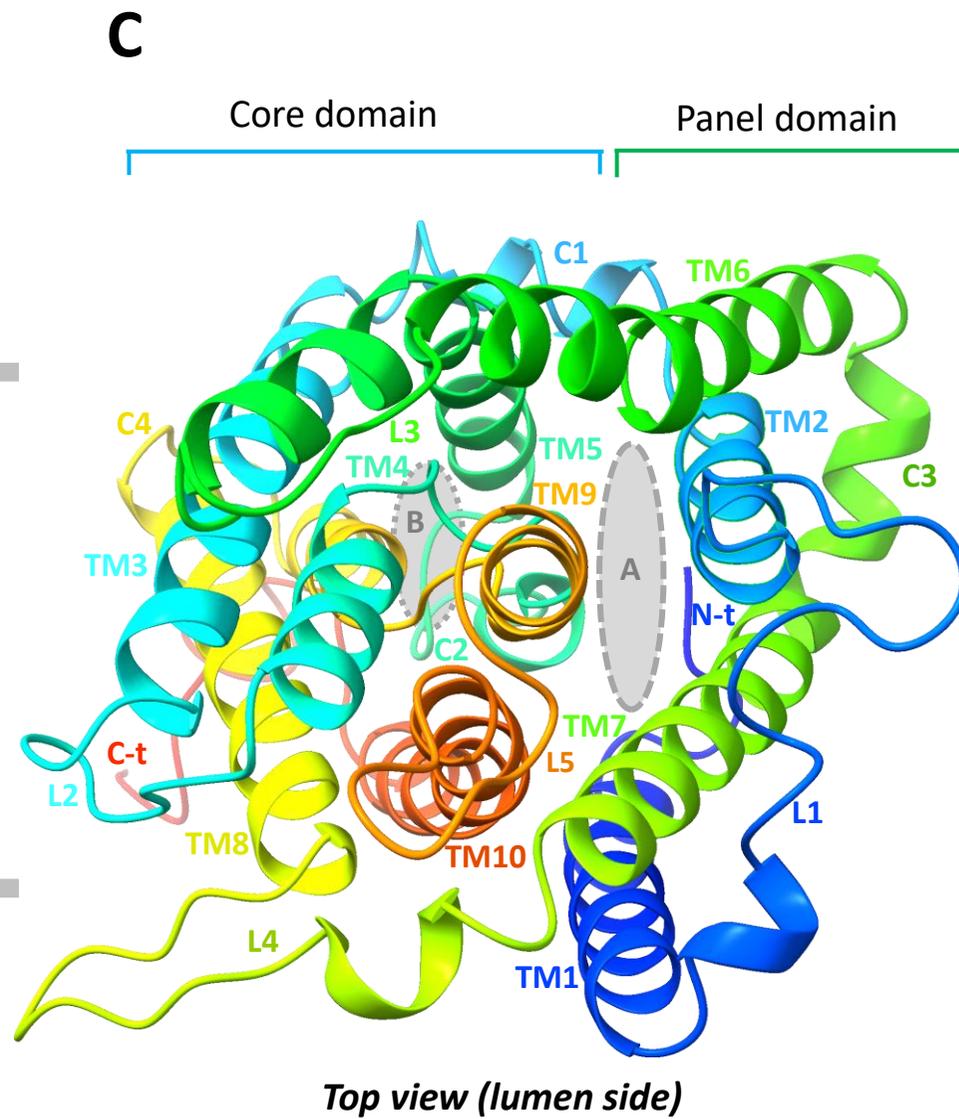
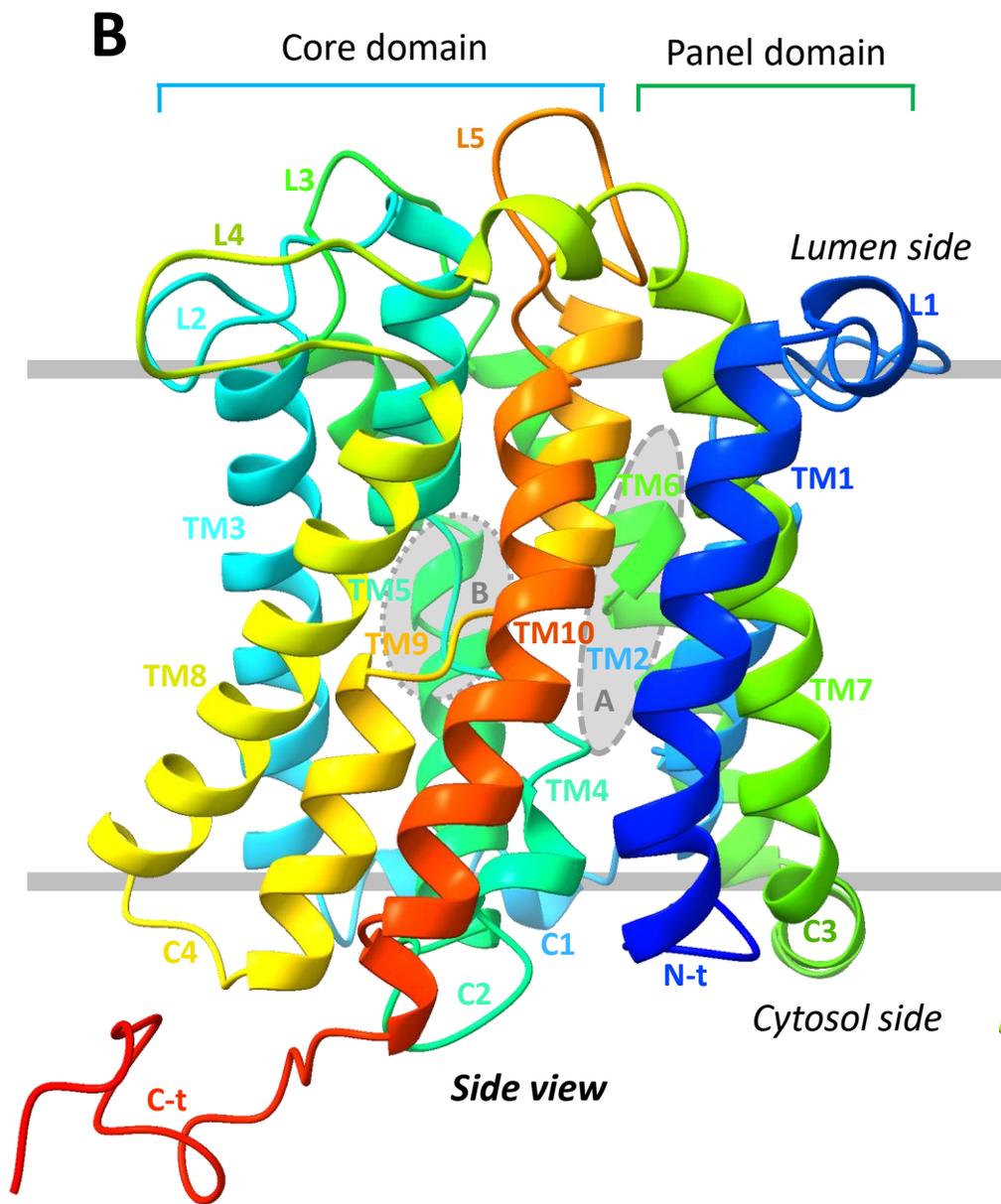
CDG, Congenital Disorders of Glycosylation; GAG, glycosaminoglycans



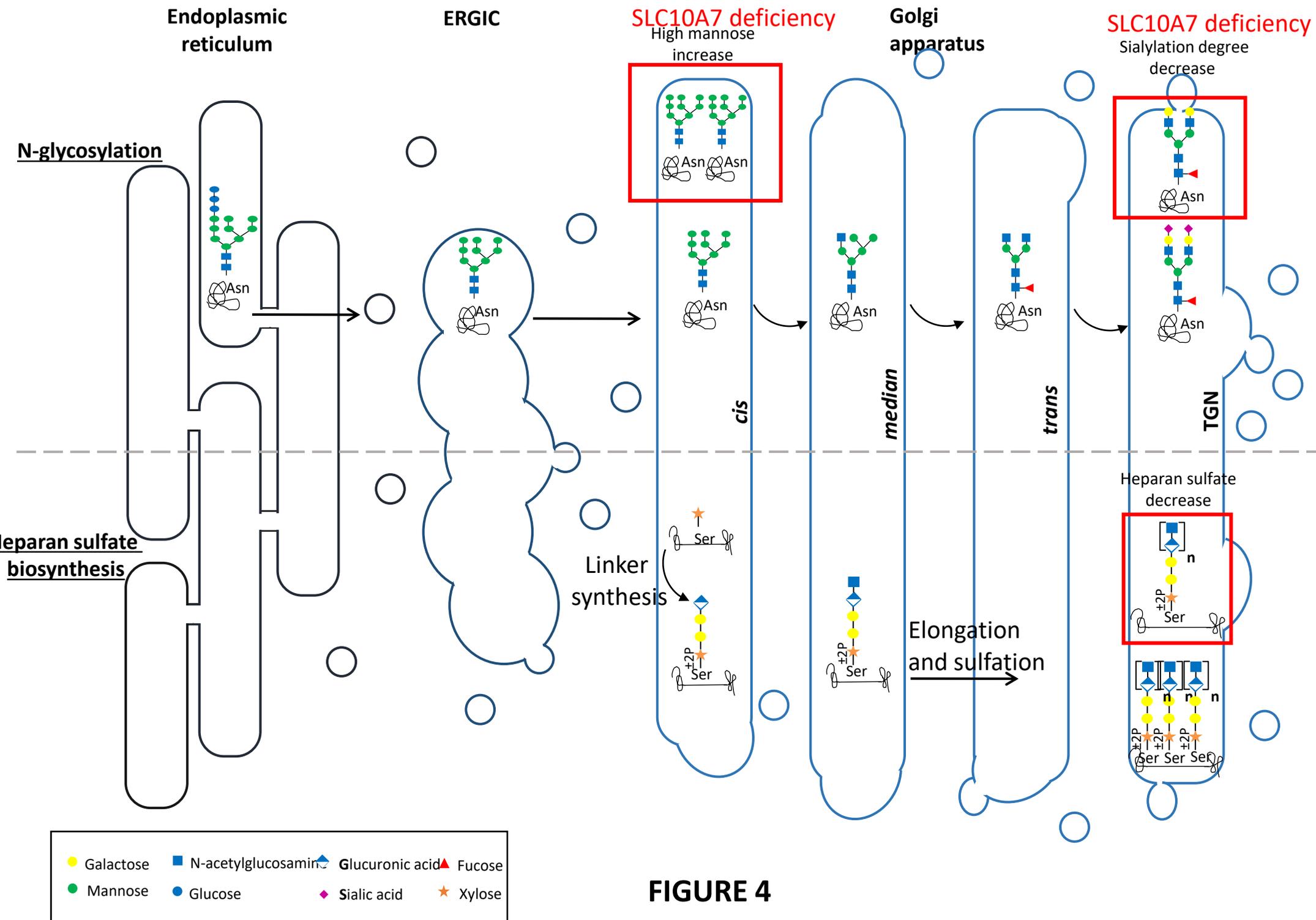
**FIGURE 1**



**FIGURE 2**







**FIGURE 4**