Review

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Congenital disorders of glycosylation in hepatology: The example of polycystic liver disease

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Autosomal dominant polycystic liver disease (PCLD) is a rare progressive disorder characterized by an increased liver volume due to many (>20) fluid-filled cysts of biliary origin. Disease causing mutations in *PRKCSH* or *SEC63* are found in ~25% of the PCLD patients. Both gene products function in the endoplasmic reticulum, however, the molecular mechanism behind cyst formation remains to be elucidated. As part of the translocon complex, SEC63 plays a role in protein import into the ER and is implicated in the export of unfolded proteins to the cytoplasm during ER-associated degradation (ERAD). *PRKCSH* codes for the β-subunit of glucosidase II (hepatocystin), which cleaves two glucose residues of $Glc_3Man_9GlcNAc_2$ N-glycans on proteins. Hepatocystin is thereby directly involved in the protein folding process by regulating protein binding to calnexin/calreticulin in the ER. A separate group of genetic diseases affecting protein N-glycosylation in the ER is formed by the congenital disorders of glycosylation (CDG). In distinct subtypes of this autosomal recessive multisystem disease specific liver symptoms have been reported that overlap with PCLD. Recent research revealed novel insights in PCLD disease pathology such as the absence of hepatocystin from cyst epithelia indicating a two-hit model for PCLD cystogenesis. This opens the way to speculate about a recessive mechanism for PCLD pathophysiology and shared molecular pathways between CDG and PCLD. In this review we will discuss the clinical-genetic features of PCLD and CDG as well as their biochemical pathways with the aim to identify novel directions of research into cystogenesis.

Keywords: Polycystic liver disease; Congenital disorders of glycosylation; Protein folding; Protein quality control; Glucosidase II; *SEC63*; *PRKCSH*.
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Abbreviations: CDG, congenital disorders of glycosylation; PCLD, polycystic liver disease; *SEC63*, SEC63 homolog (*S. cerevisiae*); *PRKCSH*, protein kinase C substrate 80K-H (80 kDa protein, Heavy chain); ADPKD, autosomal dominant polycystic kidney disease; ER, endoplasmic reticulum; BiP, binding immunoglobulin protein; ERAD, endoplasmic reticulum-associated degradation; AGE, advanced glycation end product; FGF, fibroblast growth factor; GANAB, glucosidase II alpha neutral AB.

Introduction

The identification of the genes that underlie congenital disorders of glycosylation (CDG) has led to tremendous insight into the physiology of processing and transport of glycoproteins. Often, these discoveries have had little impact in hepatology as most CDG are recessive and extremely rare, and present liver symptoms as part of a multisystem disease. Indeed, as underlined by a recent review in this Journal, hepatologists have mainly focused on disturbed glycosylation of specific glycoproteins in an effort to identify biomarkers or explain certain elements of liver pathology such as liver fibrosis [1]. The recent discovery of genes involved in glycoprotein processing as the cause of an autosomal dominant form of polycystic liver disease (PCLD) opens up the possibility for a comprehensive mapping of the role of protein glycosylation in liver pathology. The focus of this review will be on PCLD and we will discuss the role of the *SEC63* and *PRKCSH* gene products in protein glycosylation. Finally, we will highlight a number of CDG subtypes in terms of their liver pathology and discuss their pathology in relation to PCLD.

Polycystic liver disease

Clinical presentation

Autosomal dominant polycystic liver disease (PCLD) is a rare progressive disease characterized by an increased liver volume due to many (>20) fluid-filled cysts [2] (Fig. 1). The cells lining the cysts originate from bile duct epithelial cells which normally shape the biliary tree [3]. During embryonic development, bile ducts arise from the ductal plate through growth and apoptosis. In PCLD, dense complexes of intralobular bile ductules remain intact and are termed Von Meyenburg complexes [4]. These complexes develop into cysts by disconnecting from the biliary tree. Subsequent epithelial fluid secretion and growth create the mature cyst [5,6]. Symptoms arise due to continuous cyst growth and the corresponding increase in liver volume of PCLD patients [5,7]. Most patients are diagnosed between 30 and 40 years of age when they start to suffer from the increased liver volume and the pressure it exerts on other organs. They can experience

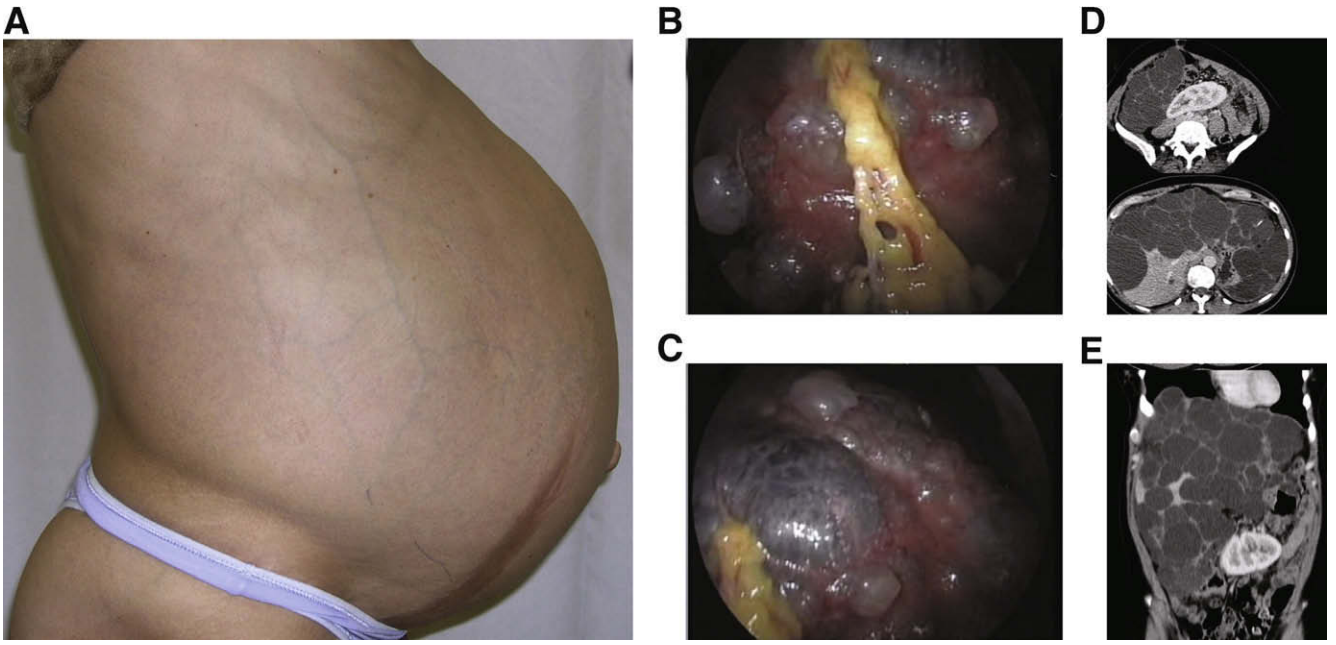

Fig. 1. Phenotype of PCLD. (A) The bulging abdomen of a severely affected patient. (B and C) Stills from a laparoscopic surgical procedure in an affected patient. In both stills, the cysts are readily identifiable. The right section of the figure shows representative transversal (D) and coronal (E) sections of a computer tomography. Note that there are multiple liver cysts but that the kidney is normal.

reduced appetite due to pressure on the stomach and shortness of breath as a result of reduced lung capacity [5]. Although the cysts disturb the normal structure and shape of the liver, the liver functions normally in these patients. There is a remarkable difference in disease severity between man and women. The latter are more severely affected and have larger liver volumes compared to their age matched male counterparts. Pregnancies (and oral anticonceptives) seem to further stimulate cyst growth in women [5], indicating that female sex hormones play a role in cyst development.

Polycystic livers are also seen in patients with autosomal dominant polycystic kidney disease (ADPKD). ADPKD is caused by mutations in either the *PKD1* or the *PKD2* gene and is characterized by the presence of multiple fluid-filled cysts in the kidney [8]. PCLD is different from ADPKD, since it is not linked to the *PKD1* or *PKD2* gene and PCLD patients do not develop kidney cysts.

So far, treatment options consist of aspiration sclerotherapy, in which cyst volume is reduced by aspiration [9]. Other options include surgical measures aimed to relieve the symptoms by mechanically reducing the number of cysts or cyst volume [10,11] as well as liver transplantation [12]. Medical therapeutic options for PCLD patients are around the corner. A recent randomized, double-blind, placebo-controlled trial of polycystic liver patients with the somatostatin analogue lanreotide, showed that this treatment led to a significant decrease in polycystic liver volume by ~4.5% [13].

Pathways involved in PCLD

Two genes are associated with PCLD, and heterozygous mutations found in these genes explain about 25% of the cases. *Protein Kinase C Substrate 80K-H* (*PRKCSH*) was identified in 2003 as the first causative gene for PCLD [14,15], while one year later *SEC63* mutations were found in different families [16]. Both genes are located in the ER and function in the early secretory route of the cell. The protein encoded by *SEC63* (SEC63) functions in protein translocation in the ER, while the protein encoded by *PRKCSH* (hepatocystin) acts in the protein folding process. Both genes are ubiquitously expressed in the human body [14–16].

SEC63

In the cell, SEC63 is located in the membrane of the endoplasmic reticulum (ER) where it is involved in protein transport across the ER membrane [17]. The main player in ER translocation is the Sec61 complex, which is also called the translocon. This heterotrimeric complex, consisting of Sec61α, Sec61β and Sec61γ, forms a transmembrane channel through which nascent proteins are translocated into the ER during protein translation (co-translational) and after being fully synthesized (post-translational). The translocon requires additional factors like SEC63 to drive the translocation and the composition of the total complex can vary (reviewed in [18]). Extensive research in yeast showed that Sec63p is required for both co-translational and post-translational translocation (Fig. 2). In the post-translational pathway the C-terminal domain of Sec63p is important for the formation and stability of the Sec62p/Sec63p/Sec71p/Sec72p complex that recruits precursor proteins to the translocon in the absence of ribosomes [19–22]. The J-domain of Sec63p interacts with the ER chaperone binding immunoglobulin protein (BiP) located in the ER and this interaction provides the driving force for protein translocation [23]. After ATP regulated interaction with the DnaJ domain of Sec63p, multiple BiP molecules bind the nascent protein and thereby prevent retrograde movement of the protein through the translocon [24–26]. The co-translational pathway also requires functional Sec63p, but here the ribosomes provide the nascent proteins and the C-terminal domain required for the stabilization of Sec62p in the post-translational pathway is

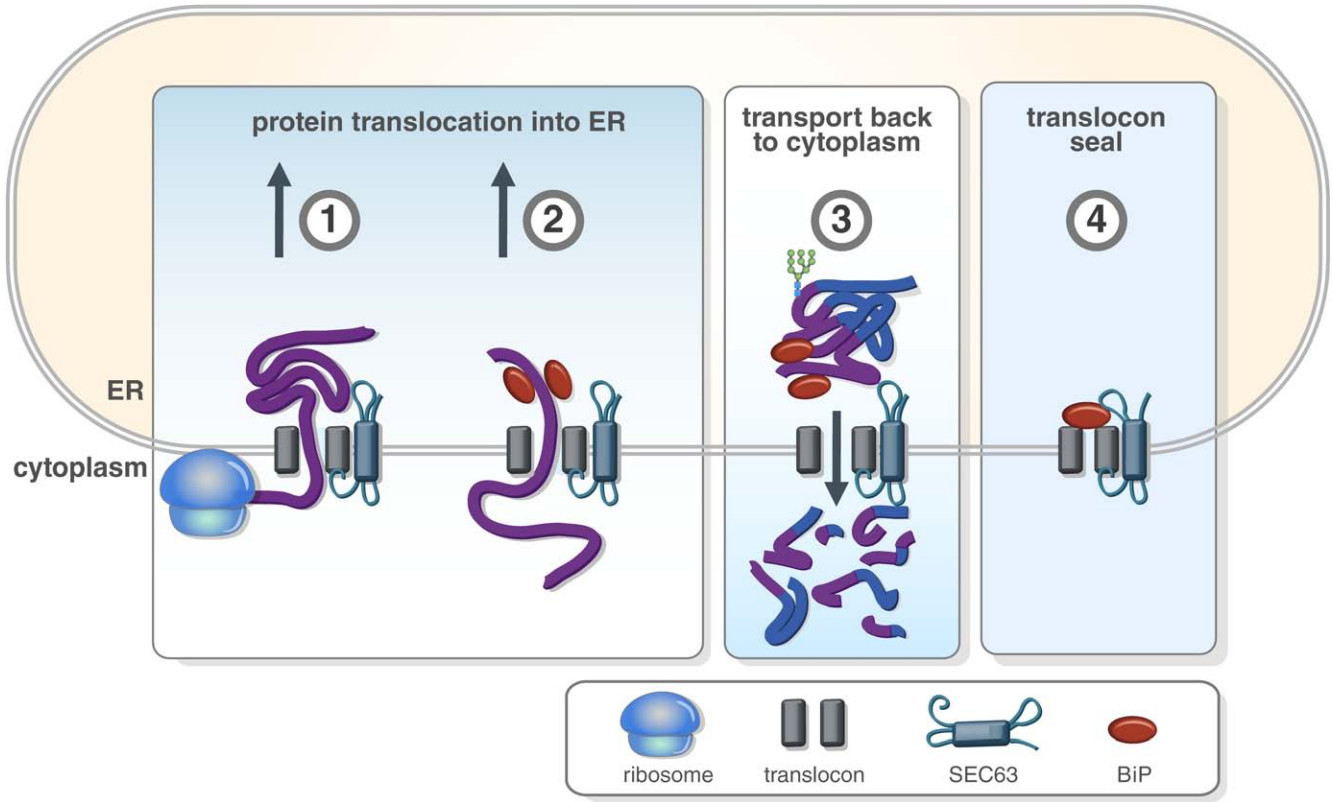

Fig. 2. Functions of SEC63. In yeast, Sec63p is involved in the structure of the translocon complex and plays an active role in protein translocation through interaction with BiP. In this way, Sec63p is required for co-translational (1) and post-translational (2) protein translocation across the ER membrane. Retrograde protein transport of misfolded proteins from the ER back to the cytoplasm also requires Sec63p (3). Furthermore, as BiP seals the translocon when it is not in use, a putative role of Sec63p is to facilitate this interaction and gate the translocon (4).

not imperative in the co-translational pathway [19,27]. Transport of proteins from the ER back to the cytoplasm occurs during ER-associated degradation (ERAD), where ER proteins prone for degradation are transported through the translocon back to the cytoplasm for degradation by the proteasome [28]. In yeast, Sec63p and BiP co-precipitate with ER-associated proteasomes and mutations in Sec63p or BiP affect degradation of unfolded proteins in the ER [29,30]. BiP recognizes hydrophobic regions of misfolded or partially assembled proteins and promotes ER retention and protein folding [31]. Furthermore, mutations in yeast *SEC63* lead to a significant reduction in the export of misfolded glycoproteins from the ER [32]. Collectively, these data show the involvement of Sec63p in protein translocation into and out of the ER (Fig. 2). In addition, Sec63p is proposed to be involved in gating the translocon by interacting with BiP, which blocks ion transport through the translocon when the channel is not in use for protein translocation [33,34].

In mammalian cells, the role of SEC63 is still unclear. It has been shown that SEC63 binds the ribosome free SEC61 complex (post-translational and ERAD), but is not associated with the co-translational ribosome–SEC61 complex [35]. Subsequent investigations showed that only a fraction of the available SEC63 was bound to SEC61 [35,36], which might implicate additional and still to be identified molecular functions for SEC63 in mammalian cells. The role of mammalian SEC63 in ERAD remains unclear because the protein complex facilitating retrograde protein transport in mammalian cells has not yet been identified.

Hepatocystin

Hepatocystin function has been associated with several subcellular processes. In COS-7 cells and in hippocampal neurons, hepatocystin was shown to interact with and regulate the activity of inositol 1,4,5-trisphosphate receptors (IP3Rs), which regulate Ca^{2+} release from the ER [37]. In the plasma membrane of numerous human cell types (including mononuclear, endothelial, renal, brain neuronal and glial cells), hepatocystin was suggested to act as an advanced glycation end product (AGE) receptor mediating cell activation, chemotaxis and cell secretion [38]. In HEK293 cells, plasma membrane hepatocystin was found to interact with the epithelial Ca^{2+} channel TRPV5 [39]. Furthermore, hepatocystin and its bovine orthologue VASAP-60, have been implicated in intracellular vesicle transport in CHO and bovine luteal cells, respectively [40,41]. Recently, hepatocystin was also found to be involved in fibroblast growth factor (FGF) signaling in human breast cancer cells and rat myoblast cells [42,43]. Taken together, hepatocystin has been implicated in a multitude of functions in several subcellular locations.

Hepatocystin is predominantly localized to the ER, where it functions as the β-subunit of the ER enzyme alpha glucosidase II [44]. This enzyme consists of two subunits, α and β, which strongly interact to form a heterodimeric protein complex [45]. Both subunits play an important role in the function of the complex [46], which is involved in glycoprotein processing (Fig. 3). In the ER, oligosaccharide structures (glycans) are attached to nas-

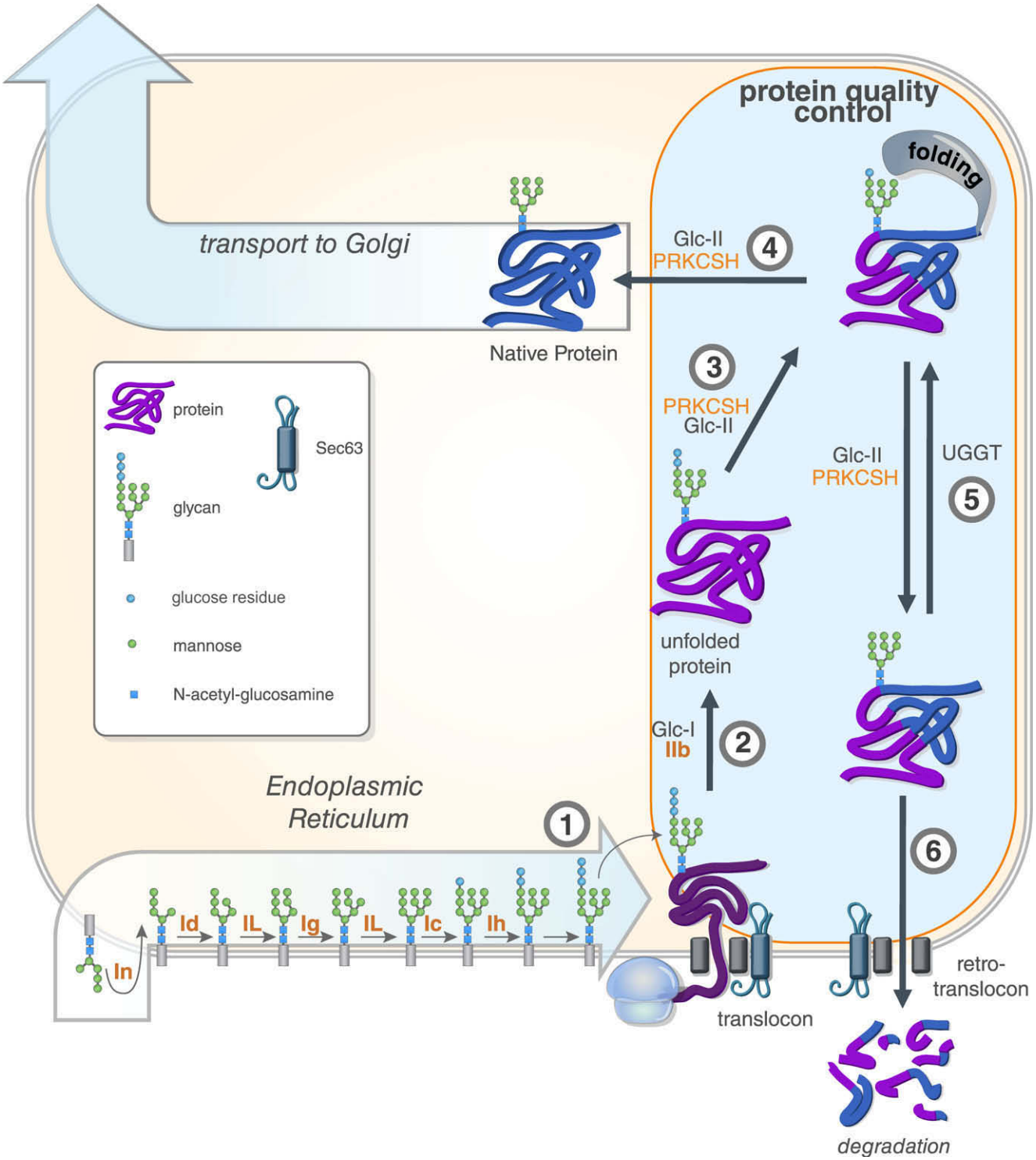

Fig. 3. Glycan modifications and protein quality control in the ER including involved genes and CDG subtypes. Formation of the glycan involves several enzymatic steps. After completion (1) the glycan is transferred by oligosaccharyltransferase (OTase) to nascent proteins entering the ER through the translocon. The first glucose residue is cleaved by glucosidase I (Glc-I) (2), the second glucose is cleaved by glucosidase II (Glc-II) (3). The resulting structure with one remaining glucose residue interacts with the ER folding machinery. Cleavage of the last glucose residue by glucosidase II releases the protein from the ER chaperones and makes the protein available for transport to the Golgi (4). Partly unfolded proteins cleaved by glucosidase II are reglucosylated by UDP-glucose glycoprotein glucosyltransferase (UGGT) to restore the interaction with the folding machinery (5). Proteins that remain unfolded are ultimately transported back to the cytoplasm and degraded by the proteasome (6). In orange the CDG subtype abbreviations and involved PCLD genes are shown.

cent glycoproteins to assist in their solubility, folding, and quality control (as reviewed by [47]). In brief, the glycans ($Glc_3Man_9GlcNAc_2$) are synthesized on the lipid dolichol, anchored in the ER membrane, and are then transferred *en bloc* to nascent proteins emerging into the ER lumen during co- or post-translational translocation. After attachment, the glycan is modified by glucosidase I and glucosidase II, which cleave specific glucose residues from the glycan. Nascent proteins depend on this glucose cleavage for their interaction with the folding chaperones in the ER. Immediately after glycan attachment, the first glucose is cleaved by glucosidase I, after which the remaining two glucoses are cleaved by the glucosidase II complex in a more controlled manner [48]. After the first two cleavages, a $Glc_1Man_9GlcNAc_2$ structure remains which is bound by the luminal ER chaperones calnexin and calreticulin. ERp57 associates with calnexin/calreticulin and mediates disulfide bond formation and folding of the nascent glycoprotein. Cleavage of the final glucose residue by the glucosidase II complex subsequently releases the nascent protein from calnexin/calreticulin [49]. Properly folded proteins are then transferred to the Golgi for further post-translational modification. When the protein is not folded properly, it is reglucosylated by UDP-glucose glycoprotein glucosyltransferase in order to enter the cycle again. Finally, misfolded proteins enter ERAD and are transported back to the cytoplasm for degradation.

The function of glucosidase II depends on both subunits. Even though the α-subunit (GANAB) holds the enzymatic activity in the complex, hepatocystin is essential for the activation and proper function of the enzyme. Yeast cell mutants, deficient for either the α-subunit or hepatocystin were both devoid of full glucosidase II activity, but viable under non-stress conditions. The hepatocystin mutants did show products of the first glucosidase II cleavage but not of the second cleavage, indicating that hepatocystin is essential for the cleavage of the glucose–mannose linkage [50,51]. In mammalian cells it was shown that hepatocystin is necessary for the solubility, retainment in the ER and activation of the α-subunit of human glucosidase [52] and that transfection of the α-subunit without its β-subunit resulted in an inactive protein [53]. The β-subunit contains a specific HDEL ER retention signal and retains the glucosidase II enzyme complex in the ER [45,52,54]. Mutation of this HDEL sequence leads to secretion of the α-subunit [52]. In addition, the number of glycans present on a protein was also shown to affect the kinetics of glucosidase II, two glycans are more efficiently cleaved compared to one [48]. The glucosidase II complex has one active site but two binding affinities and it is proposed that hepatocystin can bind the mannose branch of a glycan with its mannose-6-phosphate receptor domain causing a conformational change allowing effective cleavage of the last glucose [52,53,55].

The glycoprotein quality control system depends on proper functioning of the glucosidase II complex. Incomplete glucose cleavage results in reduced rates of protein folding, accelerated protein degradation, reduced expression of cell-surface proteins and defects in protein secretion [50,56–58]. Some proteins depend heavily on the folding machinery of the ER and cannot obtain their native conformation without glucosidase II cleavage and subsequent interaction with the ER chaperones [59,60]. In contrast, other proteins are still able to fold and reach the Golgi in the absence of glucosidase II. Additionally, alternative isoforms are described for the α-subunit and hepatocystin with specific affinity for different glycoproteins. For example, only alternatively spliced hepatocystin can associate with CD45 in hematopoietic cells [61]. This substrate specificity is interesting and alternative splicing in different cell types may explain the liver specific phenotype of PCLD.

Congenital disorders of glycosylation

N-glycosylation pathway in the ER

Protein N-linked glycosylation is an important process in the secretory pathway of the cell (Fig. 3). In the ER, it overlaps with the process of protein translocation and folding, starting with the transfer of the $Glc_3Man_9GlcNAc_2$ oligosaccharide by oligosaccharyltransferase (OTase) followed by the action of ER mannosidase I and subsequent transport to the Golgi apparatus [47]. Formation of the oligosaccharide is initiated on the cytoplasmic side of the ER membrane by addition of glucosamine to dolichol-phosphate. After extension to a dolichol linked $Man_5GlcNAc_2$ glycan, flipping of the glycan into the ER lumen is mediated by the flippase RFT1. Further extension occurs in a highly ordered manner by ER mannosyltransferases with different substrate specificity. Subsequent action of the ALG6, ALG8 and ALG10 glucosyltransferases leads to the final dolichol linked glycan $Glc_3Man_9GlcNAc_2$, which is the recognized substrate for OTase. This oligosaccharyltransferase complex, composed of eight subunits and associated with the SEC61 complex in the ER membrane [62], recognizes the terminal glucose residues and transfers the glycan onto nascent polypeptide chains containing the Asn-Xaa-Ser/Thr amino acid consensus sequence. Following glucosidase I and II action, the glycoprotein enters the folding machinery as described under 1.2 (Fig. 3). After proper folding and removal of a terminal mannose residue, the glycoprotein finds its way towards the Golgi, where processing of the glycans occurs.

Genetic defects in protein N-linked glycosylation occur in a group of autosomal recessive diseases, termed CDG. Two types can be distinguished based on the function of the gene involved. In CDG type I, the assembly of the dolichol linked $Glc_3Man_9GlcNAc_2$ oligosaccharide is affected or its transfer to nascent polypeptides by OTase. In CDG type II processing of protein linked glycans either in the ER or Golgi is affected.

Since many different enzymes are required for a proper glycosylation of proteins, CDG's are genetically heterogeneous resulting in many different clinical subtypes (Table 1). Although each individual genetic defect will lead to a similar underglycosylation of glycoproteins in CDG type I, specific clinical features seem to be present in distinct subtypes.

Presence of liver disease in CDG

Most CDG are multisystem diseases with a high mortality, characterized by neurological symptoms, gastro-intestinal problems, coagulopathy and endocrine and dysmorphic abnormalities. In most patients liver symptoms are involved. In some CDG subtypes, liver problems as hepatomegaly or hepatic fibrosis are present as key symptoms. Since hepatocystin and SEC63 are both expressed in the ER, we will focus on the liver phenotype of ER-localized CDG genes (see also Fig. 3 and Table 1).

Starting with the first defect in the lumen of the ER, mutations in *ALG3* affect $Man_5GlcNAc_2$-PP-Dol extension in CDG-Id. Diminished liver function has been reported in two patients and two siblings

Table 1. Genes and reported liver symptoms in CDG. This table describes the CDG subtype (first column) with the affected gene (second column) and the accumulating substrate of the affected enzyme (third column). The liver symptoms (fourth column) include positive findings extracted from available case reports (references in fifth column).

CDG	Gene	Oligosaccharide	Liver symptoms	Reference
In	*RFT1*	$Man_5GlcNAc_2$	hepatomegaly	[82]
Id	*ALG3*	$Man_5GlcNAc_2$	hepatomegaly bile duct malformations diminished liver function fibrosis bile lakes	[63-65]
IL	*ALG9*	$Man_6GlcNAc_2$	hepatomegaly	[66-68]
Ig	*ALG12*	$Man_7GlcNAc_2$	elevated liver enzyme activity	[69-71]
Ic	*ALG6*	$Man_8GlcNAc_2$	hepatomegaly abnormal hepatic lysosomal inclusions	[72-74]
Ih	*ALG8*	$Glc_1Man_9GlcNAc_2$	hepatomegaly multiple cystic bile ducts dilated bile ducts elevated liver enzyme activity cholestasis	[75-77]
IIb	*GLS1*	$Glc_2Man_9GlcNAc_2$	hepatomegaly dilatation and proliferation of bile ducts cholangiofibrosis steatosis cholestasis	[78]
x	*unknown*	unknown	steatosis fibrosis abnormal hepatic lysosomal inclusions	[74, 83]

with CDG-Id [63–65]. In one of these patients, hepatomegaly, bile lakes and liver fibrosis were observed. Histologically, the liver showed bile duct malformations, which were defined by the persistence of an excess of embryological bile duct structures in ductal plate configuration [63]. Mutations in the next gene, *ALG9*, lead to accumulation of $Man_6GlcNAc_2$-PP-Dol causing CDG-IL. In three CDG-IL patients, hepatomegaly was observed, while 1 patient also suffered from enlarged, cystic kidneys [66–68]. No report on liver biopsy was available. No hepatomegaly was observed in *ALG12* deficient CDG-Ig patients, in which $Man_7GlcNAc_2$-PP-Dol accumulates. Some of these patients showed an elevated activity of liver enzymes [69–71]. In CDG-Ic, $Man_9GlcNAc_2$-PP-Dol accumulated due to an ALG6 defect. CDG-Ic is generally characterized by a more neurological phenotype with epilepsy as prominent symptom, whereas liver involvement has been reported, including hepatomegaly [72,73]. Furthermore, in three CDG-Ic patients, liver biopsies revealed abnormal lysosomal inclusions in hepatocytes [74], but no bile duct abnormalities. CDG-Ih is caused by a defect in the first glucosyltransferase, *ALG8*, leading to $Glc_1Man_9GlcNAc_2$-PP-Dol accumulation. Interestingly, CDG-Ih is characterized by a severe hepato-intestinal phenotype with elevated liver enzymes and hepatomegaly [75–77]. Liver biopsy was performed in one patient, showing multiple, cystic, dilated intra- and extrahepatic bile ducts. This patient also developed cholestasis and diffuse renal microcysts [77]. The first defect after transfer of the dolichol linked glycan to the nascent protein, CDG-IIb, is caused by deficient activity of glucosidase I (*GLS1*) leading to protein linked $Glc_3Man_9GlcNAc_2$ structures. In one neonate patient, progressive hepatomegaly was observed and the patient died at 74 days. Post mortem light microscopy of the liver revealed dilatation and proliferation of the bile ducts. Furthermore, the liver displayed progressive cholangiofibrosis, steatosis, cholestasis and the appearance of bile thrombi in hepatocytes and bile ducts [78]. Finally dilated bile canaliculi have also been observed in untyped CDG patients [75].

Discussion

Glycosylation is an important cellular process and defects in glycosylation can lead to a large variety of cellular defects and human disorders. *PRKCSH*, one of the two genes associated with PCLD, is directly involved in the N-glycosylation machinery in the ER and plays a role in protein folding. Most CDG are recessive diseases where patients carry mutations in both alleles of the affected gene. In contrast, PCLD is an autosomal dominant disease with a heterozygous germ line mutation, affecting only one allele of the affected gene. It was shown that in patients with a heterozygous *PRKCSH* mutation hepatocystin is normally expressed in most parts of the liver but hepatocystin was absent from bile duct cells lining the cysts [44]. This suggests that the wild-type *PRKCSH* allele is lost from the cyst epithelium through a somatic second hit mutation (Fig. 4). Examples of a cellular recessive mechanism in related dominant diseases are hereditary multiple

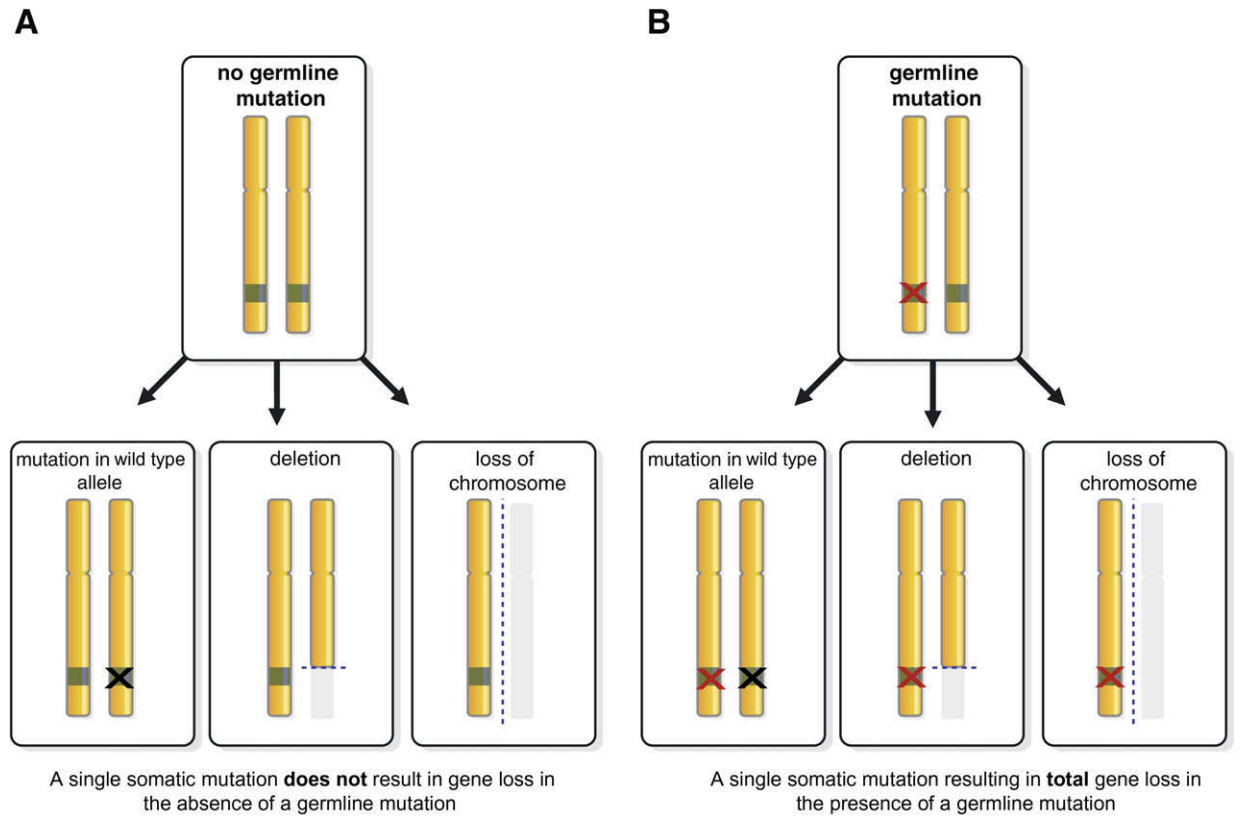

Fig. 4. Loss of wild-type allele through second hit mutation. Somatic mutations can occur sporadically throughout life and affect single cells. As most genes are presented by two functional alleles, a somatic mutation in one allele will often not affect the expression of this gene in the cell. Total gene loss only occurs if in the same cell both alleles would acquire a mutation (A). However, if an inherited mutation (germ line mutation) inactivates one allele, all the cells in the body are left with only one functional allele of that specific gene. A single somatic mutation affecting the remaining allele can cause loss of gene expression in these cells (B). As a result, the chance of total gene loss is much higher in someone with a germline mutation. How the gene loss will affect the cell depends on the gene and the type of cell involved. In the case of PCLD loss of *PRKCSH* or *SEC63* from a bile duct cell appears to result in the formation of a liver cyst.

exostoses [79] and ADPKD [80]. Complete loss of *PRKCSH* from the cyst epithelium is expected to affect the function of glucosidase II and as a consequence glycosylation and protein folding in these cells. This seems to be confirmed by the finding that in cyst epithelial cells, specific membrane proteins are retained in the ER whereas these proteins are normally distributed in hepatocystin expressing biliary cells [56].

PCLD can also be caused by mutations in *SEC63*, a gene which is thought to be involved in protein transport into and out of the ER. Mutations in *SEC63* or *PRKCSH* result in the same disease which could indicate that the genes are functionally related. Because in a large percentage of the patients no mutation in *PRKCSH* or *SEC63* can be identified, it is likely there is at least one more (yet unknown) gene involved in the development of PCLD [81]. Identification of this gene may provide the missing link between the function of SEC63 and hepatocystin. Although the number of CDG patients per subtype is limited, as is the number of liver biopsies performed, the few available reports clearly show an overlap of specific liver features with PCLD. The liver biopsies in CDG show dilated bile ducts (CDG-Ib, CDG-Id, CDG-Ih and CDG-IIb) and liver cysts (CDG-Ih). CDG is recognized early in life during childhood, whereas PCLD diagnosis is made between 30 and 40 years. Thus, it seems that development of liver cysts is much faster in CDG compared to PCLD, where liver cysts are not detected before 30 years of age. An explanation for this observation may be that in the CDG patients the gene is lost in all cells whereas in PCLD only some cells lose the gene through a second hit mutation.

The biochemical defect of at least two of these subtypes (CDG-Ih and CDG-IIb) shows considerable overlap with the protein translocation and folding process in PCLD (Fig. 3). The dolichol linked glycan that accumulates in CDG-Ih ($Glc_1Man_9GlcNAc_2$) is similar to the protein linked glycan in *PRKCSH* deficient cells, assuming the second hit model is valid. It has been shown that incomplete glycans can be transferred from dolichol to proteins, but at reduced efficiency compared to the fully synthesized glycans, and that the abnormal glycan can then affect the folding and degradation of the protein in the ER [68]. In CDG-Ih cells the $Glc_1Man_9GlcNAc_2$ structure may be transferred in small amounts from dolichol to proteins in the absence of fully synthesized $Glc_3Man_9GlcNAc_2$, thereby resembling protein glycosylation in *PRKCSH* deficient cells. Likewise, defective glucosidase I in CDG-IIb might interfere in the same biochemical process, e.g. protein folding or ERAD.

Concluding remarks and future perspectives

Much has been learned about PCLD over the last few years, but still many issues are unclear. By comparing PCLD with CDG we hope to open a whole new discussion in the pathogenesis of PCLD

and gain insight in the underlying molecular mechanisms as well as to obtain possibilities for future treatments. Future studies should first confirm the two-hit model. Subsequently, it will be important to investigate the cell type specific effects of hepatocystin and SEC63 and determine how their absence relates to possible glycan and protein accumulations. These studies could also lead to the identification of other genes involved in PCLD pathogenesis, thereby facilitating mutation analysis and screening in affected families.

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