

Polycystic liver disease is a disorder of cotranslational protein processing

Joost P.H. Drenth^{1,2}, Jose A. Martina², Rolf van de Kerkhof¹, Juan S. Bonifacino² and Jan B.M.J. Jansen¹

¹The Department of Medicine, Division of Gastroenterology and Hepatology, University Medical Center St. Radboud, Nijmegen, 6500 HB, The Netherlands

²Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

Autosomal-dominant polycystic liver disease (PCLD) is a rare disorder that is characterized by the progressive development of fluid-filled biliary epithelial cysts in the liver. Positional cloning has identified two genes that are mutated in patients with polycystic liver disease, *PRKCSH* and *SEC63*, which encode the β -subunit of glucosidase II and Sec63, respectively. Both proteins are components of the molecular machinery involved in the translocation, folding and quality control of newly synthesized glycoproteins in the endoplasmic reticulum. Most mutations are truncating and probably lead to a complete loss of the corresponding proteins and the defective processing of a key regulator of biliary cell growth. The finding that PCLD is caused by proteins involved in oligosaccharide processing was unexpected and implicates a new avenue for research into neocystogenesis, and might ultimately result in the identification of novel therapeutic drugs.

Liver cysts as a genetic disorder

Isolated liver cysts are common in the general population. Hereditary polycystic livers, arbitrarily defined as having more than 20 cysts, however, are infrequent and appear to occur with or without polycystic kidneys [1]. In autosomal-dominant polycystic kidney disease type 1 (ADPKD-1) and type 2 (ADPKD-2), for example, patients have polycystic kidneys and often a polycystic liver. ADPKD arises as a consequence of mutations in the *PKD1* and *PKD2* genes, which encode the proteins polycystin 1 and 2, respectively [2]. In autosomal-dominant polycystic liver disease (PCLD), patients have a polycystic liver but lack polycystic kidneys. In 2003 and 2004, positional cloning efforts identified mutations in at least two separate genes that are responsible for PCLD, *PRKCSH* and *SEC63*. These findings provide a new entry point for research, because the biological function of the proteins encoded by *PRKCSH* and *SEC63* are different from those associated with ADPKD. This article discusses the implications of the identification of these genes for the pathogenesis of PCLD.

Phenotype of PCLD

PCLD occurs in families from Northern European and American descent and is diagnosed by radiological studies that confirm the presence of liver cysts [3]. The disease usually remains asymptomatic until it manifests between the age of 40 and 60 years. Abdominal pain is the most prominent feature and arises due to local pressure on the stomach and duodenum. The enlarged polycystic liver might also cause nausea, vomiting and early satiety, with consequent weight loss, anorexia, shortness of breath, discomfort and sleep apnoea. Sudden abdominal pain is rare and, when it occurs, is caused by cystic rupture or intracystic hemorrhage. Depending on the severity of the phenotype, abdominal distension is present with a palpable hepatic mass. In rare cases, portal hypertension ensues because of inferior vena cava compression and venous outflow obstruction [4]. In general, laboratory tests remain normal and the functional protein producing capacity of liver is preserved, even in advanced cases. There is a striking difference in clinical presentation between the sexes. Females have a more severe phenotype with more and larger symptomatic cysts at an earlier age. Despite these considerations, in most instances, PCLD is asymptomatic, as documented by a series of 49 patients diagnosed with polycystic liver disease in which the majority (77%) denied symptoms [1]. Studies in ADPKD have indicated a vascular co-morbidity with aneurysms of cerebral and coronary artery vessels, and mitral valve prolapse [5]. In PCLD, echocardiographic studies have suggested that structural mitral leaflet abnormalities are more frequent in these patients, compared with unaffected family members (20.4% versus 0%) [1]. A major caveat is that this study was performed before the advent of genetic testing, which might have confounded the results because the absence of a molecular diagnostic test made it difficult to distinguish between affected and unaffected family members.

Therapy

PCLD is refractory to medical treatment and (at least to date) the discovery of causative genes has not changed this perspective [3]. A more complete understanding of the molecular pathogenesis, coupled to the development of

Corresponding author: Drenth, J.P.H. (Joost.PHDrenth@CS.com).

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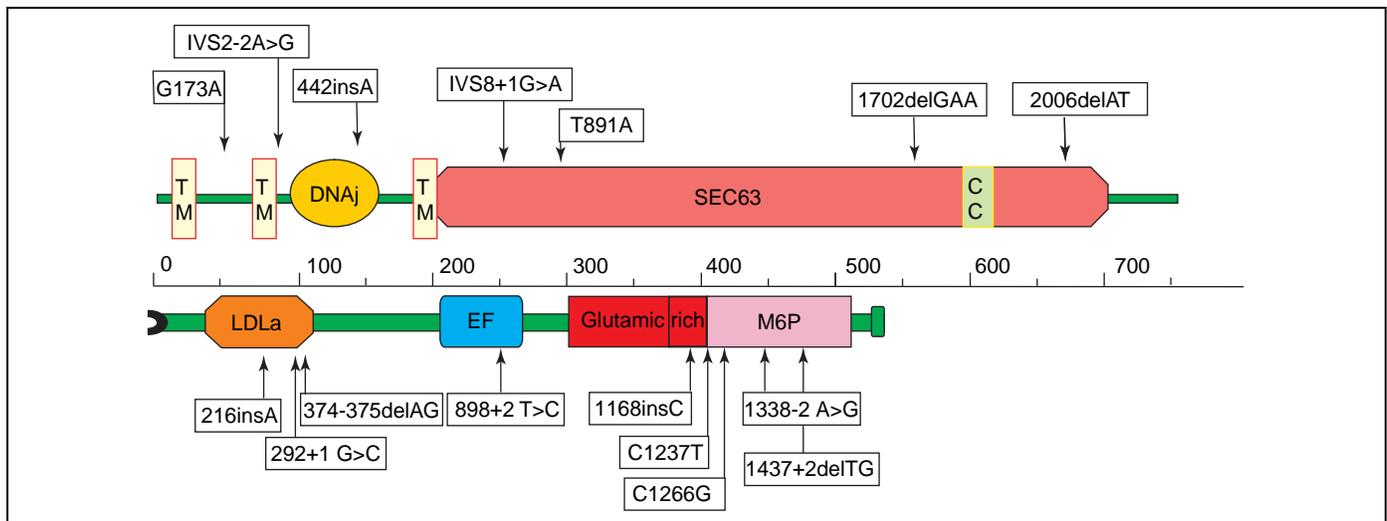


Figure 1. Schematic representation of hepatocystin and SEC63. The amino acid sequences were analysed for protein domains or motifs. SEC63 is depicted above the scale (which indicates the amino acid number) and hepatocystin is shown below. The positions of SEC63 and PRKCSH mutations that have been associated with PCLD are superimposed on the structures. Note that for both proteins, mutations are distributed along the sequence. Abbreviations: TM, transmembrane span; CC, coiled coil; LDLa, low-density lipoprotein receptor domain A; EF, EF-hand calcium binding domains; MPR, mannose 6-phosphate receptor domain. Adapted from [18], with permission from The American Gastroenterological Association.

animal models for the disease will certainly aid the identification of new therapeutic compounds.

Clinically, the majority of patients do not need treatment, but in cases with a significant mass effect and clinical symptoms, therapeutic options should be explored. The aims of therapy are to excise or obliterate the cyst completely or partially to achieve the relief of symptoms and prevent recurrences. The management of PCLD can be regarded as a step-up approach and covers the spectrum from less to more invasive procedures. In milder cases, percutaneous guided drainage of cysts can be considered. To prevent recurrence, aspiration of cyst fluid is followed by the subsequent infusion of a sclerosant, usually ethanol. Ethanol destroys the lining epithelium of the cyst and causes fibrotic obliteration. In experienced hands, success, which is defined as the definitive obliteration of individual cysts, occurs in more than 70% of cases. Surgical treatment of advanced cases includes the fenestration of selected cysts [6]. This technique exposes the cysts by laparotomy or laparoscopy and de-roofs the cyst, enabling free drainage in the peritoneal cavity. Complications include bile leak, ascites, bleeding and infections. Although this approach results in an appreciable reduction of hepatic volume, recurrences that necessitate repeated procedures do occur and complications are not infrequent [7]. Several factors predict an unsatisfactory outcome, such as inadequate de-roofing, fenestration of deep-seated cysts and cysts in the posterior segments of the liver. Segmental liver resection is reserved for symptomatic PCLD that is refractory to radiological or surgical cyst decompression. It results in the significant long-term and sustained reduction of symptoms but is associated with considerable perioperative morbidity, and mortality occurs.

A recent report suggested an alternative therapy for PCLD, consisting of the embolization of hepatic arterial branches with microcoils, and this approach resulted in a halving of the liver volume in a single patient [8]. Liver

transplantation is an option for symptomatic patients in whom other therapeutic efforts have failed. Most experience with liver transplantation has been gained in ADPKD patients with concomitant polycystic liver disease and data from a limited number of patients (<10) indicate that it is feasible for this indication [9].

Genetics of PCLD

In 2000, genetic linkage analysis in two large families located the PCLD locus to a 12.5 cM interval on chromosome 19p13.1–13.2 [10]. In 2003, the first mutated gene that was associated with PCLD was independently identified by two laboratories using a classic forward genetic approach [11,12]. One study of four large Dutch PCLD pedigrees confirmed the localization of the gene and refined it to a 2.1 cM region [11]. Chromosome 19 is particularly gene rich, and identification of the gene involved in PCLD required screening by the direct sequencing of 78 genes. A heterozygous mutation (1338–2A>G) at the splice acceptor site of intron 15 of the protein kinase C substrate (*PRKCSH*) gene was found in affected individuals from three families, whereas a different splice-donor site mutation of intron 4 (292+1G>C) was seen in another family (Figure 1). In another study, mutations in *PRKCSH* were independently identified using a combination of denaturing high-performance liquid chromatography (DHPLC) heteroduplex analysis and direct sequencing [13]. This study examined 36 affected individuals from six separate families and detected six different *PRKCSH* mutations. Three families were large enough to enable the demonstration of disease segregation with the mutations. The mutations detected so far are seen throughout the gene, from exon 3 to exon 16. The predicted effect of all pathogenic variants is a premature termination of translation. Indeed, RT-PCR experiments have proven this to be the case for at least three splice-site mutations (292+1G>C, 1338–2A>G and 1437+2delTG). Altogether, at least nine different

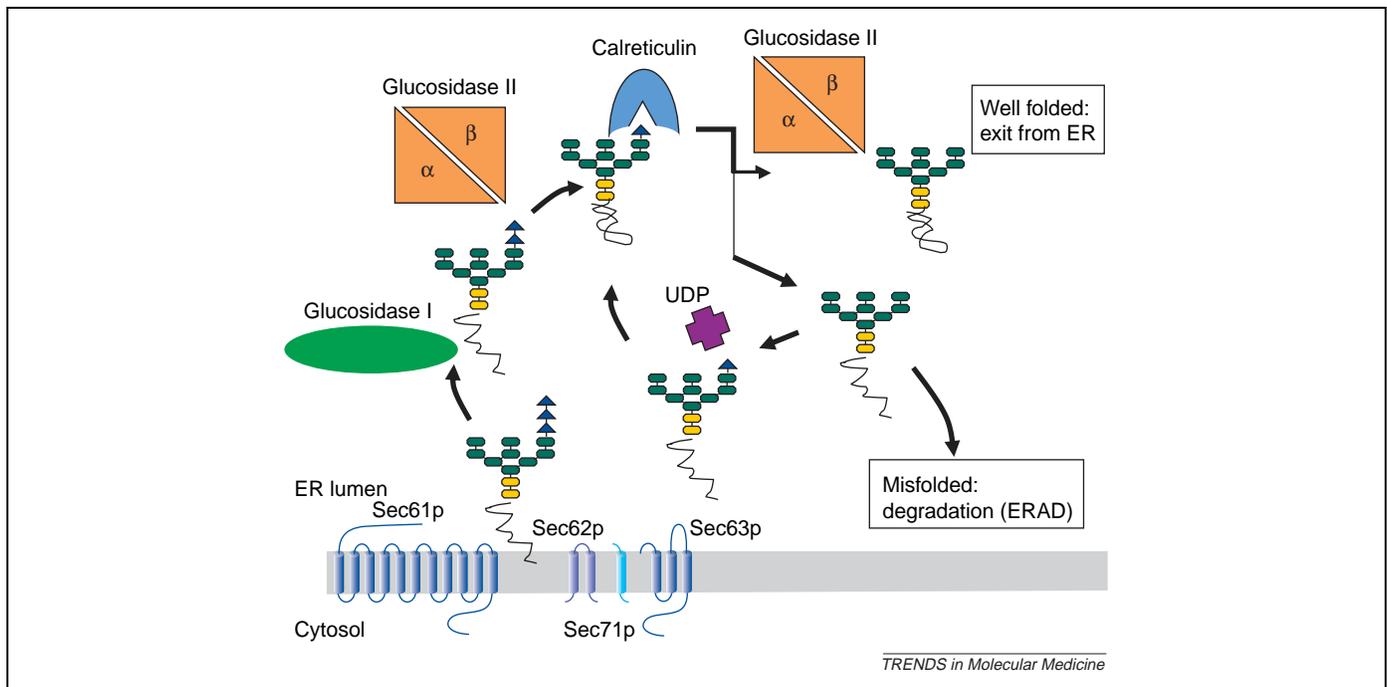


Figure 2. The role of Sec63 and hepatocystin in glycoprotein handling in the endoplasmic reticulum (ER). Protein translocation into the ER is facilitated by the translocon. The Sec61 translocon associates with a second oligomeric membrane protein complex, the Sec62–Sec63 complex. This complex contains a cytoplasmic signal sequence receptor site that binds to newly synthesized glycoproteins. The luminal J domain of Sec63 facilitates recognition by ER chaperones at the luminal exit site of the translocon. After transfer of a glycoprotein into the ER lumen through the translocon, three glucoses (blue triangles) are trimmed away by glucosidase I and II, respectively, and terminal mannoses (green hexagons) by one or more different ER mannosidases (not shown). Glucosidase II consists of a catalytic α -subunit and a non-catalytic β -subunit (hepatocystin). This generates a monoglucosylated glycoprotein that binds to calnexin and/or calreticulin, two ER chaperones that are specific for monoglucosylated core oligosaccharides (note that only calreticulin is shown). The protein is thereby exposed to another folding factor, ERp57, a thiol oxidoreductase, and associates with both chaperones. Cleavage of the remaining glucose by glucosidase II terminates the interaction with calnexin and calreticulin. On their subsequent release, correctly folded glycoproteins can exit the ER. If the glycoprotein is not folded at this time, the oligosaccharides are re-glucosylated by UDP-glucose-glycoprotein glucosyltransferase (depicted as UDP), which places a single glucose back onto the glycan and thereby promotes a renewed association with calnexin and calreticulin. If the protein is permanently misfolded, they are recognized by a quality control receptor and targeted for re-translocation through the translocon (ERAD).

truncating *PRKCSH* mutations have been identified in PCLD [14]. Haplotype analysis suggests that a founder effect might account for the same germline mutation in several apparently unrelated families. Indeed, two mutations probably arose independently in two separate regions in the Netherlands, whereas another mutation is common in the Finnish population.

Genetic heterogeneity and a second PCLD locus

Bidirectional sequencing of the complete *PRKCSH* gene excluded pathogenic variants in two large Finnish PCLD kindreds [15], thus providing firm evidence for genetic heterogeneity [14]. This led to another positional cloning effort that identified a second locus for the disease. This study screened 66 patients from unrelated families for *PRKCSH* mutations. A mutation in this gene was excluded in 57 patients, including those from ten families with multiple affected members. These ten families were used in a genome-wide study that detected a locus on chromosome 6q21 [13]. Within this interval, *SEC63* appeared to be a candidate gene and seven heterozygous mutations were found in this gene among eight patients with PCLD from a total of five families, including both Finnish families (Figure 1). Similar to *PRKCSH*, the majority of mutations (6/7) is predicted to result in premature chain termination and is consistent with a loss of function. In contrast to *PRKCSH* mutations, however, each family had their own specific *SEC63* mutation, with the W58X mutation being the exception

because it was observed in two unrelated patients with PCLD. This indicates that these mutations arose independently in the different PCLD families. The fact that this large sequencing effort excluded *PRKCSH* and *SEC63* mutations in 49 patients with PCLD suggests that at least one other locus is involved in the disease [13].

Molecular diagnostic considerations

Studies in PCLD do not support mutational hot spots in *PRKCSH* or *SEC63*, and apparently there is no direct effect of genotype on the resultant phenotype. Nevertheless, severe PCLD was found to be more frequent among *PRKCSH*-positive patients (67%) compared with *SEC63*-positive patients (42%) [14]. Although little is known about the mutation rate of *PRKCSH* and *SEC63* in the PCLD population, a few points can be made. Previous studies indicated that *PRKCSH* mutations were more likely in patients with either severe polycystic liver disease (arbitrarily >20 liver cysts) or those with a positive family history, and it seems reasonable to direct diagnostic efforts to patients that match these criteria [14]. It must be noted that the presence of isolated renal cysts in these patients does not exclude PCLD *per se*, because *PRKCSH* mutations were found in patients with polycystic livers who had up to ten renal cysts [14]. Furthermore, one might consider starting screening *PRKCSH* in Dutch or Finnish patients and focus in particular on the founder mutations. In other populations, it is probably unimportant whether to screen first for

SEC63 or *PRKCSH*. For example, in one series of 66 patients, *PRKCSH* mutations were found in 14%, whereas *SEC63* mutations were detected in 12%. [13] In view of the many different mutations spread over *PRKCSH* and *SEC63*, direct sequencing of the separate exons is currently the most prudent strategy.

The PCLD proteins

Hepatocystin

The *PRKCSH* gene encodes a previously described human protein termed protein kinase C substrate 80K-H [16]. Given its role in the pathogenesis of PCLD, this protein has been renamed hepatocystin. Theoretical analysis predicts that hepatocystin contains a signal peptide for translocation across the ER membrane, a low-density lipoprotein receptor domain class A (LDLa) domain, two EF-hand domains, a glutamic-acid-rich region, a manose-6-phosphate receptor domain and a conserved C-terminal HDEL amino acid sequence for endoplasmic reticulum (ER) retention (Figure 1). Despite its initial identification as a protein kinase C substrate, it has been demonstrated that hepatocystin is the non-catalytic β -subunit of glucosidase II, an ER-resident enzyme that is involved in carbohydrate processing and quality control of newly synthesized glycoproteins (Figure 2). Because the catalytic α -subunit of glucosidase II lacks known ER retention sequences, it is probable that the β -chain (hepatocystin) serves as a localization subunit. It is also possible that the β -subunit is needed for the proper folding of the α -subunit [17]. Recent immunofluorescent data indicate that hepatocystin resides in the ER [18]. Immunoprecipitation-recapture studies show that normal hepatocystin associates with the α -subunit of glucosidase II. The truncating 1338–2A>G *PRKCSH* mutation results in a protein that does not assemble with the α -subunit and is not retained in the ER, but is secreted into the medium from the apical and basolateral plasma membranes. Consequently, mutant hepatocystin is undetectable in PCLD liver tissue by western blot experiments, and protein levels of normal hepatocystin and glucosidase II α -subunit are greatly reduced. A recent study suggested that hepatocystin might also be involved in the calcium-dependent control of the epithelial calcium-channel transient receptor potential cation channel V5 (TRPV5), and this study identified several domains (EF hands, the glutamic-acid-rich region and the HDEL sequence) that are important for its function [16].

Sec63 protein

Human Sec63, which is also affected in PCLD, is an 83-kDa protein [19] that is predicted to span the ER membrane three times and contain a luminal N-terminus, a cytoplasmic C-terminus with a coiled-coil region and a luminal DnaJ domain between the second and third transmembrane span (Figure 1) [13]. Two membrane-spanning complexes have been shown to mediate the translocation of nascent or newly synthesized polypeptides across the ER membrane of eukaryotic cells. One of these complexes is composed of the Sec61p, Sss1p and Sbh1p (the Sec61 complex) proteins (yeast nomenclature), whereas the other contains two essential proteins (Sec62p

and Sec63p) and two non-essential proteins (Sec71p and Sec72p). Most studies have been performed in yeast, in which each complex has been shown to mediate a different type of translocation. The Sec61-containing complex mediates co-translational translocation, in which the recognition of signal peptides by the signal recognition particle (SRP) and the SRP receptor results in the targeting of ribosome-associated nascent polypeptides to the Sec61 complex [20]. Alternatively, the Sec62–Sec63-containing complex mediates the posttranslational translocation of full-length precursors in a manner dependent upon cytosolic chaperones. Mutations in any of the four genes encoding components of the Sec62–Sec63 complex cause the cytosolic accumulation of full-length protein precursors [20]. In particular, the luminal J-domain of Sec63 protein is crucial for the interaction with a chaperone protein, Kar2p (the yeast BiP homologue), and its mutation results in a major defect in posttranslational translocation [21]. However, only one of the mutations detected in PCLD targets the J-domain, whereas others are spread evenly across the Sec63 protein (Figure 1) [13].

ER glycosylation and quality control

Both proteins implicated in PCLD (Sec63 and hepatocystin) are involved in translocation through the ER membrane and in the oligosaccharide processing of newly synthesized glycoproteins (Figure 2). Carbohydrates can be covalently attached to asparagine (N-glycans) residues of polypeptides. N-linked glycans are important for protein folding, covering antigenic domains and protecting the peptide backbone against proteases. Nascent polypeptides elongating on ribosomes are translocated across the ER membrane through translocon complexes. The translocon is a multi-functional complex that is involved in regulating the interaction of ribosomes with the ER, as well as regulating the translocation and integration of membrane proteins in the correct orientation. N-linked glycans, as presynthesized oligosaccharides, are co-translationally transferred *en bloc* to proteins in the lumen of the ER by the action of the oligosaccharyltransferase that is adjacent to translocons. The processing of N-glycan starts immediately after their addition, with the trimming of terminal glucose moieties by the sequential actions of the enzymes glucosidase I and II. Glucosidase I deficiency is associated with a severe phenotype of dysmorphia, generalized hypotonia and early death (Box 1) [22]. The mono-glucosylated peptide binds to calnexin and/or calreticulin, which are molecular chaperones that prevent the aggregation, premature degradation and export of the incompletely folded chains from the ER. They also expose the polypeptides to Erp57, an enzyme with thiol-disulfide oxidoreductase activity, which provides assistance during disulfide bond formation. Glucosidase II is responsible for dissociating the substrate glycoprotein from calnexin or calreticulin by hydrolysing the glucose from the monoglucosylated core glycan, following which the complexes dissociate and the proteins are free to leave the ER. If the glycoprotein is not folded at this time, the oligosaccharide chain is re-glucosylated by ER UDP-glucose glycoprotein glucosyltransferase and the protein

Box 1. Glucosidase I deficiency

The loss of glucosidase II leads to PCLD, but defects in glucosidase I are associated with a different, more severe phenotype. Glucosidase I removes the distal α -1,2-linked glucose residue of the glycan structure. The resulting intermediate is then further modified by glucosidase II and several ER-resident mannosidases and glycosyltransferases. A glucosidase I defect is extremely rare and has been described as an autosomal-recessive disorder in a single neonate. This patient was a compound heterozygote for two glucosidase I gene mutations (R486T and F652L) that led to a near-complete glucosidase I deficiency [22]. Phenotypically, there was dysmorphism and generalized hypotonia and the clinical course was complicated by hypoventilation, feeding problems and seizures. Interestingly, there was also a liver phenotype with development of hepatomegaly and dilation of bile ducts complicated by cholangiofibrosis and macrovesicular steatosis. This patient died prematurely.

re-enters the cycle. Alternatively, ER quality control mechanisms ensure that proteins missing the proper tertiary structure are retrotranslocated into the cytoplasm for degradation by the ubiquitin–proteasome pathway.

Concluding remarks

The identification of *PRKCSH* and *SEC63* as the genes implicated in PCLD has provided a better understanding in the pathogenesis of this disease. Unlike the proteins implicated in ADPKD, hepatocystin and Sec63 are not located in cilia (Box 2), but are involved in the ER processing of glycoproteins.

N-linked glycans are a prominent feature of glycoproteins and are used as specific tags or recognition signals [23]. This establishes PCLD as a disorder of protein biogenesis. The processing of N-linked glycans in the ER requires the entry of the polypeptide into the ER (with Sec63 of particular importance) and subsequent recognition of the oligosaccharide chain by glucosidase I and II. The inhibition or genetic disruption of glucosidase II in tissue cultures probably leads to less efficient glycoprotein folding and secretion, partial breakdown of the quality control system, with incompletely folded proteins being secreted, and induction of the unfolded protein response [17,24]. If the folding and maturation process fails, the protein is not transported to its final destination in the cell and is eventually degraded. Protein misfolding can be detrimental to the cell and contributes to the disease mechanism in other disorders, such as in autosomal-dominant familial neurohypophyseal diabetes insipidus, in which mutations result in the retention of proteins in the ER because they fail to fold and/or dimerize properly [25]. The physiological role of the proteins hepatocystin and Sec63 are clear indicators that ER glycosylation and quality control are central to the pathology of PCLD. Because both Sec63 and hepatocystin are involved in the transport and handling of glycoproteins in the ER, it is likely that a mutation in these proteins leads to the mishandling of certain client proteins. In this respect, it seems unlikely that mutant Sec63 and hepatocystin directly cause neocystogenesis, although it cannot currently be ruled out. It is more likely that mutant sec63 and hepatocystin affect a single protein or a set of proteins that mediate biliary cell

Box 2. Cilia as localization for cyst associated proteins

Several lines of evidence point to cilia as the anatomical converging point in ADPKD. Cilia are located at the apical surface of most cell lines. Hepatocytes are among the notable exceptions. Cilia are particularly well developed in the mammalian kidney and each tubular cell (except for intercalated cells) usually possesses a single organelle known as a 'primary cilium'. The proteins defective in ADPKD, polycystin 1 and 2, have been localized to the cilia [26] and co-localise with ciliary proteins of the microtubular machinery in the stem and basal body of the primary cilium [27]. Primary cilia are required for the induction of intracellular calcium spikes in response to physiological levels of fluid shear stress. The stress-induced calcium response in kidney epithelial cells is mediated by polycystin-1 and polycystin-2, which preferentially localize to the base of the primary cilium. Flow-induced bending of the cilium results in conformational changes of polycystin-1 that transduce the mechanical signal into a chemical response through the activation of associated polycystin-2, which acts as a calcium channel [28]. In contrast to the situation in ADPKD, the proteins involved in PCLD, hepatocystin and SEC63, are not known to be localized to cilia. This would implicate a non-ciliary pathway in PCLD.

growth and proliferation. As a result of the loss of either Sec63 and/or hepatocystin, these proteins are not translocated (*SEC63* mutants) or are misfolded, less stable and susceptible to degradation (*PRKCSH* mutants) (Box 3). Further research should focus on the identification of the

Box 3. Outstanding questions

Despite the discovery of two genes involved in PCLD, little is known about how mutations in the corresponding proteins actually cause the disease. Specifically, it is unclear why the disease arises at a later age and why its phenotype is restricted to the liver.

Late onset and two-hit hypothesis

Only a few hepatic cysts are detectable in affected individuals in the first four decades of life, but hundreds of individual cysts can be present at a later age. The heterozygous nature of the disease implicates that, at most, there is a 50% loss of function of the encoded protein, but this is a constant feature and does not explain the late onset of PCLD. This is reminiscent of the situation in ADPKD, in which there is also a gradual increase in the number of renal and hepatic cysts. It has been hypothesized that ADPKD cysts arise as a result of a cellular recessive two-hit mechanism [29]. According to this scheme, carriers of a germline mutation acquire a somatic mutation on the second allele of the cell. This cell gains a proliferative advantage and displaces the cells that did not take a second hit. Eventually, this leads to lumen formation and the subsequent development of a cyst. In the case of a germline *PRKCSH* mutation in PCLD, the second hit could involve a complete loss of the other allele or a somatic *PRKCSH* or *SEC63* mutation on the other allele [30]. Similarly, it is possible that the somatic hits affect genes implicated in ADPKD, such as *PKD1* and *PKD2*.

Liver phenotype

Northern blot analysis showed the ubiquitous expression of both *PRKCSH* and *SEC63* transcripts in human tissues, including liver, heart and kidney, whereas western blot analysis detected high levels of hepatocystin in virtually every tissue tested [18]. Despite this wide distribution of both *PRKCSH* and *SEC63* transcripts, the disease is restricted to biliary epithelium. Most probably, mutations in *PRKCSH* and/or *SEC63* affect the processing and/or secretion of a minor subset of all glycoproteins. This would be in agreement with observations that there are no gross changes in the glycosylation status of most major glycoproteins detected with a panel of lectins [18]. Thus, the disease probably results from the defective processing of a specific regulator of biliary cell proliferation in the liver.

client proteins that are affected in PCLD and how this can cause biliary proliferation. A better understanding of the molecular mechanisms involved in these pathways, particularly hepatocystin and Sec63, will open new horizons for therapeutic intervention.

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References

- Qian, Q. *et al.* (2003) Clinical profile of autosomal dominant polycystic liver disease. *Hepatology* 37, 164–171
- Peters, D.J. and Breuning, M.H. (2001) Autosomal dominant polycystic kidney disease: modification of disease progression. *Lancet* 358, 1439–1444
- Everson, G.T. *et al.* (2004) Polycystic disease of the liver. *Hepatology* 40, 774–782
- Torres, V.E. (1995) Polycystic liver disease. *Contrib. Nephrol.* 115, 44–52
- Gieteling, E.W. and Rinkel, G.J. (2003) Characteristics of intracranial aneurysms and subarachnoid haemorrhage in patients with polycystic kidney disease. *J. Neurol.* 250, 418–423
- Yang, G.S. *et al.* (2004) Combined hepatic resection with fenestration for highly symptomatic polycystic liver disease: A report on seven patients. *World J. Gastroenterol.* 10, 2598–2601
- Ishibashi, K. *et al.* (2004) Is fenestration a safe treatment for adult polycystic liver disease? A report of refractory complications. *Hepatogastroenterology* 51, 1165–1167
- Ubara, Y. *et al.* (2004) Intravascular embolization therapy in a patient with an enlarged polycystic liver. *Am. J. Kidney Dis.* 43, 733–738
- Ueda, M. *et al.* (2004) Living-donor liver transplantation for polycystic liver disease. *Transplantation* 77, 480–481
- Reynolds, D.M. *et al.* (2000) Identification of a locus for autosomal dominant polycystic liver disease, on chromosome 19p13.2-13.1. *Am. J. Hum. Genet.* 67, 1598–1604
- Drenth, J.P. *et al.* (2003) Germline mutations in *PRKCSH* are associated with autosomal dominant polycystic liver disease. *Nat. Genet.* 33, 345–347
- Li, A. *et al.* (2003) Mutations in *PRKCSH* cause isolated autosomal dominant polycystic liver disease. *Am. J. Hum. Genet.* 72, 691–703
- Davila, S. *et al.* (2004) Mutations in *SEC63* cause autosomal dominant polycystic liver disease. *Nat. Genet.* 36, 575–577
- Drenth, J.P. *et al.* (2004) Abnormal hepatocystin caused by truncating *PRKCSH* mutations leads to autosomal dominant polycystic liver disease. *Hepatology* 39, 924–931
- Tahvanainen, P. *et al.* (2003) Polycystic liver disease is genetically heterogeneous: clinical and linkage studies in eight Finnish families. *J. Hepatol.* 38, 39–43
- Gkika, D. *et al.* (2004) 80K-H as a new Ca²⁺ sensor regulating the activity of the epithelial Ca²⁺ channel transient receptor potential cation channel V5 (TRPV5). *J. Biol. Chem.* 279, 26351–26357
- D'Alessio, C. *et al.* (1999) Genetic evidence for the heterodimeric structure of glucosidase II. The effect of disrupting the subunit-encoding genes on glycoprotein folding. *J. Biol. Chem.* 274, 25899–25905
- Drenth, J.P. *et al.* (2004) Molecular characterization of hepatocystin, the protein that is defective in autosomal dominant polycystic liver disease. *Gastroenterology* 126, 1819–1827
- Skowronek, M.H. *et al.* (1999) Molecular characterization of a novel mammalian DnaJ-like Sec63p homolog. *Biol. Chem.* 380, 1133–1138
- Rothblatt, J.A. *et al.* (1989) Multiple genes are required for proper insertion of secretory proteins into the endoplasmic reticulum in yeast. *J. Cell Biol.* 109, 2641–2652
- Willer, M. *et al.* (2003) An *in vitro* assay using overexpressed yeast SRP demonstrates that cotranslational translocation is dependent upon the J-domain of Sec63p. *Biochemistry* 42, 7171–7177
- de Praeter, C.M. *et al.* (2000) A novel disorder caused by defective biosynthesis of N-linked oligosaccharides due to glucosidase I deficiency. *Am. J. Hum. Genet.* 66, 1744–1756
- Helenius, A. and Aebi, M. (2004) Roles of N-linked glycans in the endoplasmic reticulum. *Annu. Rev. Biochem.* 73, 1019–1049
- Zhang, J.X. *et al.* (1997) Quality control in the secretory pathway: the role of calreticulin, calnexin and BiP in the retention of glycoproteins with C-terminal truncations. *Mol. Biol. Cell* 8, 1943–1954
- Christensen, J.H. *et al.* (2004) Impaired trafficking of mutated AVP prohormone in cells expressing rare disease genes causing autosomal dominant familial neurohypophyseal diabetes insipidus. *Clin. Endocrinol. (Oxf.)* 60, 125–136
- Yoder, B.K. *et al.* (2002) The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J. Am. Soc. Nephrol.* 13, 2508–2516
- Nauli, S.M. *et al.* (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* 33, 129–137
- Cantiello, H.F. (2003) A tale of two tails: ciliary mechanotransduction in ADPKD. *Trends Mol. Med.* 9, 234–236
- Pei, Y. (2001) A 'two-hit' model of cystogenesis in autosomal dominant polycystic kidney disease? *Trends Mol. Med.* 7, 151–156
- Pei, Y. *et al.* (1999) Somatic PKD2 mutations in individual kidney and liver cysts support a 'two-hit' model of cystogenesis in type 2 autosomal dominant polycystic kidney disease. *J. Am. Soc. Nephrol.* 10, 1524–1529

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