

Somatic Hits in Polycystic Liver Diseases

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Abstract

Polycystic Liver Disease (PLD) encompasses a number of disorders with the development of multiple cysts distributed throughout the liver either focally or equally. Hepatic cysts are fluid-filled cavities lined by benign epithelium. PLD is the major phenotype of isolated Polycystic Liver Disease (PCLD) and Autosomal Dominant Polycystic Kidney Disease (ADPKD).

The molecular principles in carcinogenesis indicate that there is an accumulation of multiple (somatic) mutations. This concept assumes that presence of a germline mutation ('first hit') in an inherited disorder requires a 'second hit' at the somatic level for cyst development to occur. The second hit is the rate-limiting step and results in somatic inactivation of the normal allele.

Studies have identified secondary, somatic hits in human liver cyst tissues in PCLD and ADPKD. Inactivation of both copies in PLD is demonstrated through somatic mutations or loss of heterozygosity (LOH). The frequency of somatic mutations varies between genes and genomic disorders. Genetic studies detected LOH in 9% and somatic mutations in 8-29% in ADPKD derived hepatic cysts. In PCLD, almost ~80% of hepatic cysts from *PRKCSH* carriers had completely lost the *PRKCSH* gene.

There is important clinical heterogeneity among PLD patients. Differences in phenotypical expression may be explained by age, gender and environment, but also modifier genes or inactivating somatic events may play key roles. This review will give an overview of the data gained from genetic studies in liver cyst tissues from PCLD and ADPKD patients in relation to the clinical manifestations.

Keywords: Second hit hypothesis; Somatic mutation; LOH; Loss-of-function; PLD; PCLD; ADPKD

Abbreviations: ADPKD: Autosomal Dominant Polycystic Kidney Disease; AE2: Anion Exchanger; AQP1: Aquaporin-1; cAMP: Adenosine 3',5'-Cyclic Monophosphate; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; ER: Endoplasmic Reticulum; Epac: Exchange protein directly activated by cAMP; ERK: Extracellularly Regulated Kinase; LOH: Loss Of Heterozygosity; MAPK: Mitogen-Activated Protein Kinase; PC1,PC2: Polycystin-1-2; PCLD: Isolated Polycystic Liver Disease (autosomal dominant); PKA: Protein Kinase A; PKD1,PKD2: Polycystic Kidney Disease-1, -2; PLD: Polycystic Liver Diseases; *PRKCSH*: Protein Kinase C substrate 80K-H (80-kDa protein, Heavy chain); *SEC63*: *Saccharomyces cerevisiae* homolog 63; SR: Secretin Receptor; SSTR: Somatostatin Receptor

Introduction

Polycystic liver disease (PLD) comprises a group of diverse congenital disorders that have presence of multiple hepatic cysts in common. There are 2 major conditions that possess this benign phenotype, isolated polycystic liver disease (PCLD) and autosomal dominant polycystic kidney disease (ADPKD) [1,2]. Both Mendelian disorders are autosomal dominantly inherited and share similar liver features [3]. PCLD is characterized exclusively by presence of hepatic cysts, and polycystic livers are the most common extrarenal manifestation in ADPKD [4]. Development of polycystic kidneys is a key feature in ADPKD, but also cardiovascular manifestations, intracranial aneurysms, pancreatic cysts and renal complications with end-stage renal disease may be present [1,3].

The origin of hepatic cystogenesis probably starts in early embryological phase with biliary tree development. The formation of the ductal plate is needed for development of healthy bile ducts. In genetic disorders leading up to PLD this process is compromised, hence the term ductal plate malformation [5]. It is likely that the nidus

needed for cyst formation (microcysts) is already present in childhood of germline carriers. These microcysts are undetectable by routine radiological methods, but may develop later in life [4].

Following Knudson 'second-hit' hypothesis, it was proposed that cysts arise as a result of a second mutational event. Patients carrying a germline mutation (the 'first hit' in a PLD gene) are prone for cyst development. However, the single mutated allele does not necessarily lead to hepatic cysts, but a second somatic event is required for individual cysts to develop [6].

In this short review we present a comprehensive overview of somatic mutations and genetic mechanisms that have been associated with hepatic cystogenesis in PLD.

Genetic Background

PCLD and ADPKD are distinct disorders and associated with germline mutations in separate genes. About ~25% of PCLD patients carry a bonafide gene mutation in the *PRKCSH* gene or *SEC63* gene [7-10]. Both protein products, hepatocystin and *Sec63p*, are involved in protein folding, quality control and transduction in the endoplasmic reticulum [11]. Almost all ADPKD patients carry a pathogenic germline

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PKD1 gene (~85%) or PKD2 gene (~15%) mutation [12-14]. The corresponding protein products polycystin-1 (PC-1) and polycystin-2 (PC-2) function as a mechanosensory receptor-channel complex at the primary cilium for calcium influx [1].

The primary cilium is an extending organelle at the plasma membrane of the bile duct epithelium cell (cholangiocyte). Pathophysiological processes are functionally engaged with this structure by detection of the luminal flow. Defective polycystin expression contributes to decreased intracellular calcium and increased cAMP levels [15]. The combination of these abovementioned mechanisms and overexpression of PKA and Epac cause cholangiocyst hyperproliferation through the mitogen-activated protein kinase/extracellularly regulated kinase (MAPK/ERK) pathway and cyst fluid hypersecretion in PLD [16]. Current somatostatin analogue treatment affects secretin-mediated pathways (Figure 1) [17].

General Principle in Tumorigenesis

In 1953 Nordling was the first to propose the multi-mutation hypothesis for the origin of cancer. His theory stated that accumulation of DNA mutations in a cell as a consequence of normal cell proliferation, environmental exposure and increasing age results in carcinogenesis [18]. Clinical and epidemiological observations prompted Knudson to reformulate this hypothesis with the example of dominantly inherited and acquired retinoblastoma [19]. He proposed a 'second hit' hypothesis which implicates that a second mutation is required in addition to a 'first hit' for tumor development.

Since then, several studies in common malignant diseases, particularly those caused by tumor suppressor genes such as ovarian and colon cancer, have produced data consistent with the mutational origin of cancer. This concept is refined and proof is provided that occurrence

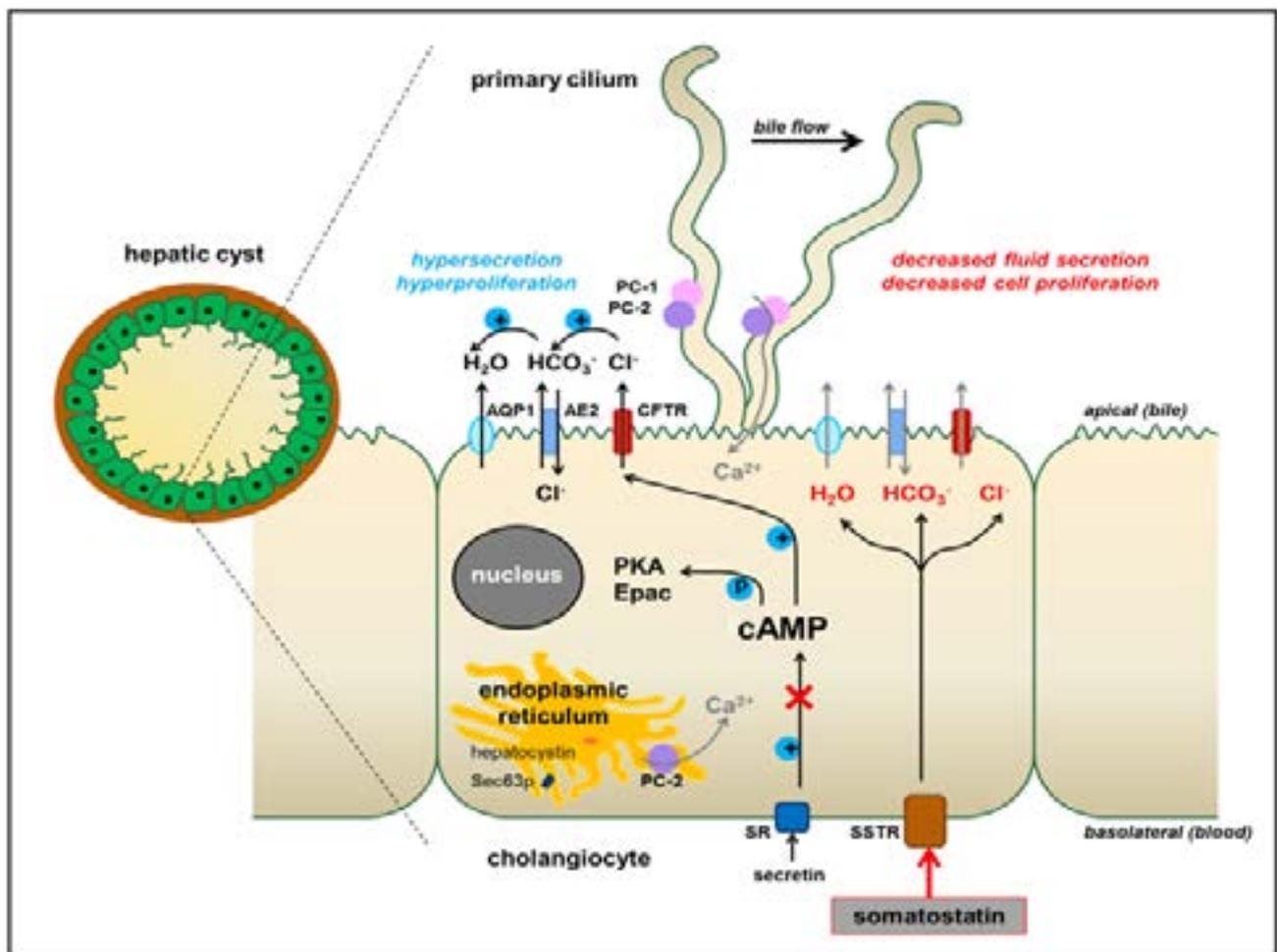


Figure 1: Somatostatin treatment decreases cholangiocyte hyperproliferation and fluid hypersecretion in PLD. Hepatic cysts are delineated by bile duct epithelium cells. The primary cilium at the plasma membrane is continuously exposed to luminal bile flow. Mechanical flow rate is sensed by bending the organelle. Under healthy conditions this stimulates calcium signaling and inhibits forskolin-stimulated cAMP signaling intracellularly. PC-2 co-localizes with PC-1, and PC-2 is a calcium channel expressed at the endoplasmic reticulum. Cholangiocytes possess numerous transporters and exchangers. Basolateral secretin stimulates intracellular cAMP signaling and bicarbonate fluid rich secretion by activation of apical CFTR chloride channel. Subsequently, secreted bicarbonate drives passive AQP1-mediated water transport to the extracellular compartment. This figure illustrates a defective molecular mechanism in PLD. Decreased intracellular calcium and accumulation of second messenger cAMP contributes to hepatic cystogenesis [15]. Phosphorylation of PKA and Epac are associated with increased MAPK/ERK signaling in PLD. In response to this activated pathway fluid secretion and cell proliferation is facilitated [16]. Somatostatin acts via SSTR-2 somatostatin receptors to increase cGMP which inhibits secretin-mediated cAMP synthesis. Secretin-stimulated chloride, bicarbonate and water secretion are inhibited and absorption is induced by somatostatin analogues (in red).

of 2 (somatic) ‘hits’ may be sufficient for tumor development in sporadic malignant diseases [20,21]. The result from both mechanisms is biallelic inactivation through loss of the wild-type copy. Second hits are usually small deletions or insertions resulting in a truncated protein, but large deletions at the gene locus with Loss Of Heterozygosity (LOH) are also seen [20-24]

Identification of Somatic Inactivation in ADPKD and PCLD

Subsequent to the identification of the first causative *PKD1* gene for ADPKD, researchers have speculated about presence of somatic mutations that would lead to the inactivation of the second *PKD1* allele [12]. In order to address this issue, methods have been developed to isolate cyst-lining epithelial cells to access DNA from single cysts. These studies confirmed the second hit hypothesis in renal cyst epithelium from *PKD1* affected patients [23,25,26].

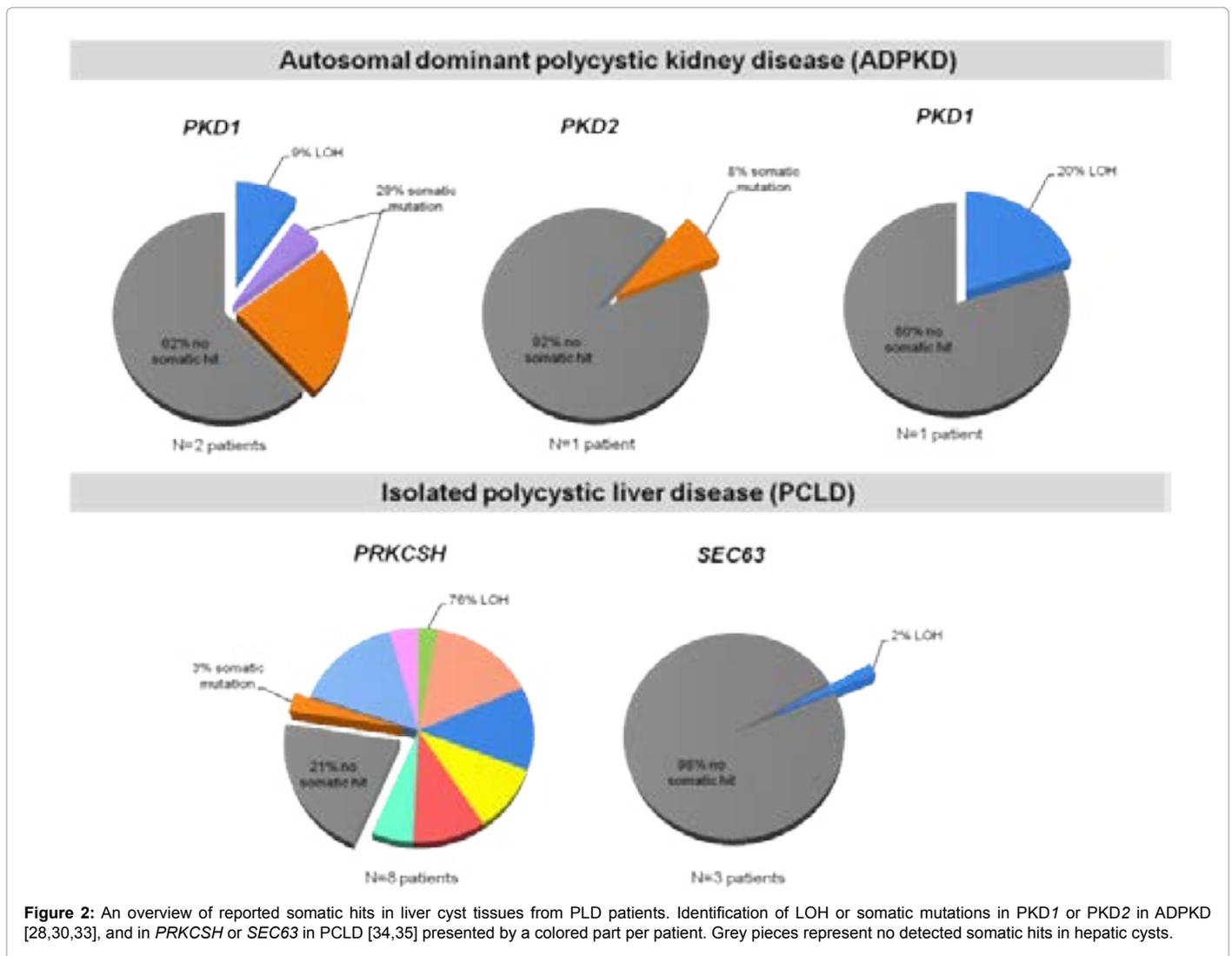
Kidney

Genetic analyses using microsatellite markers established loss of the healthy *PKD1* allele in 4-24% of renal cysts from *PKD1* affected ADPKD patients. Somatic mutations were reported in 17% of renal

cysts from a *PKD1* mutation carrier [23,25-28]. Likewise, renal cysts from ADPKD patients harboring a *PKD2* germline mutation had LOH at the *PKD2* locus with a frequency of 0-12% and (milder) somatic *PKD2* mutations were present in 15-64% of renal cysts [29-32]. In addition, these analyses showed that the majority of single renal cysts in ADPKD presented epithelial cell populations derived from 1 cell. These experiments fueled the idea that cysts consist of a monoclonal cell population build from a single cell affected by 2 hits [23,25].

Liver

Analysis of hepatic tissues (21 cysts) from 2 ADPKD patients that were *PKD1* mutation carriers showed that the other allele was lost in both cases because of LOH (9%) or somatic mutations (29%) (Figure 2 and Table 1) [33]. Another study in an ADPKD patient unlinked to *PKD1*, the healthy *PKD1* allele in hepatic cyst epithelium was lost in 20% because of LOH [28]. The situation was different in a *PKD2* carrier. Somatic hits were detected in 1/13 (8%) cysts using classic intragenic and microsatellite marker analyses [30]. By contrast, direct Sanger sequencing studies at the somatic level in 71 cysts from PCLD patients (*PRKCSH* carriers) detected a LOH incidence of 76% at the *PRKCSH* locus [34]. This is different from *SEC63* mutation carriers where *SEC63* LOH was present in 2% [35].



Identical to the situation in common tumors, the type of somatic mutations in PLD can be substitutions, small deletions or insertions leading to a missense change or premature stop mutation (Table 2) [30,33,34]. PCLD patients harboring a *PRKCSH* germline mutation are highly at risk for somatic mutational inactivation. Loss of the *PRKCSH* protein influences progression of hepatic cystogenesis. Therefore, it is possible that *PRKCSH* acts as a tumor suppressor gene.

Somatic Inactivation in Sporadic Hepatic Cysts

Sporadic cysts may arise as an incidental finding or as an acquired hepatic cyst from a traumatic or an infectious condition. Simple hepatic cysts occur in about ~10% of the general population and are frequently asymptomatic [6]. However, sporadic cysts may increase in volume and cause pressure on surrounding organs.

Germline mutations are required for cyst formation in congenital PLD, but are usually absent in individuals with a sporadic cyst. It is likely that loss of PLD genes occurs through somatic inactivation. Depending on the target tissue, loss of ADPKD alleles may lead to renal as well as hepatic cysts.[30,33]. This mechanism has not (yet) been identified in PCLD patients.

A loss-of-function model could be hypothesized as a common molecular mechanism in PLD. Different mutation types, deletions or LOH of a region including a PLD gene has been stated, but LOH may be also the result of errors during meiosis I or II. In acquired uniparental disomy an individual received 2 chromosomes from 1 parent. This

abnormal normal haplotype leads to disease in case this allele is non-functional. The consequence of this gene conversion is that the patient carries a copy number neutral LOH. For example, LOH in combination with these mechanisms are detected in other benign tumors such as neurofibromatosis, but also in malignancy [22,24].

Protein Expression Levels in Liver Cyst Tissue

Immunohistochemistry analyses of gene products may indicate the presence or the expression level of proteins. Patients harboring a truncating heterozygous germline mutation usually have a second, wild-type allele. Following the second hit hypothesis in PLD, this wild-type allele will be lost and no protein expression will be detected by staining experiments. Indeed, studies of liver cyst tissues from patients with a germline *PRKCSH* mutation demonstrated that the *PRKCSH* protein was absent in cyst epithelium, but *Sec63p* was expressed [34,36]. Vice versa, the cyst that harbored somatic LOH of *SEC63* showed reduced expression of the *Sec63p*, but positive *PRKCSH* staining [35]. These findings suggest that both PCLD protein products do not interact.

On the contrary, genetic studies in human liver cyst tissues from PKD2 affected patients [29,32] found that the majority represented polycystin-2 expression and equal polycystin-1 expression [37]. Molecular studies showed that polycystins interact by coiled-coil domains to form multimeric complexes. Interaction studies in ADPKD showed that polycystin-1 is a regulator of polycystin-2 activity in liver and kidneys [38]. This argues that *PKD* mutation carriers share the predominant phenotype of polycystic kidneys.

PLD sample ID.	Germline mutation(s)*	Predicted protein effect	Somatic mutation*	Predicted protein effect	Ref.
ADPKD1 JHU415	PKD1 c.12378C>G	p.Tyr4126X	PKD1 c.12551insGC	p.His4185Argfs*13	[33]
ADPKD1 JHU452	PKD1 c.7165T>C PKD1 c.9047A>G	p.Gln3016Arg	PKD1 c.8900C>G PKD1 c.10050+2del20 PKD1 c.8558T>C PKD1 c.7567G>T PKD1 c.8733del16	p.Ser2967X aberrant splicing p.Phe2853Ser p.Glu2523X p.Asp2912Argfs*77	[33]
ADPKD2 UT1500	PKD2 c.2152insA	p.Asn720Lysfs*5	PKD2 c.710-8del19	aberrant splicing	[30]
PCLD patient 1	<i>PRKCSH</i> c.1341-2A>G	aberrant splicing	<i>PRKCSH</i> c.224T>C <i>PRKCSH</i> c.1499G>A	p.Phe75Ser p.Cys500Tyr	[34]

*TranscriptID. *PRKCSH* (NM_002743.2); PKD1 (L33243.1); PKD2 (NM_000297.2)

Table 1: Somatic mutations with the predicted effect on protein level in liver cyst tissue from PLD patients. Types of somatic hits included 5 substitutions, 3 deletions and 1 insertion mutation at the similar gene locus (GRCh37-hg19). These mutations resulted in pathogenic mis-sense changes or truncated protein products.

Study group	Phenotype	Patients germline mutation(s)*	Hepatic cysts (n)	Ref.
Watnick et al. 1998	ADPKD1	JHU415. c.12378C>G	12	[33]
	ADPKD1	JHU452. PKD1 c.7165T>C and PKD1 c.9047A>G	9	
Pei et al. 1999	ADPKD2	UT1500. PKD2 c.2152insA	13	[30]
Badenas et al. 2000	ADPKD1	3. Germline mutation not available	15	[28]
Janssen et al. 2011	PCLD	1. <i>PRKCSH</i> c.1341-2A>G	14	[34]
	PCLD	2. <i>PRKCSH</i> c.1341-2A>G	12	
	PCLD	3. <i>PRKCSH</i> c.1341-2A>G	9	
	PCLD	4. <i>PRKCSH</i> c.1341-2A>G	9	
	PCLD	5. <i>PRKCSH</i> c.292+1G>C	5	
	PCLD	6. <i>PRKCSH</i> c.292+1G>C	13	
	PCLD	7. <i>PRKCSH</i> c.292+1G>C	7	
	PCLD	8. <i>PRKCSH</i> c.1341-2A>G	2	
Janssen et al. 2012	PCLD	1. <i>SEC63</i> c.1703_1705delAAG	34	[35]
	PCLD	2. <i>SEC63</i> c.1703_1705delAAG	4	
	PCLD	3. <i>SEC63</i> c.958G>T	14	

*TranscriptID. *PRKCSH* (NM_002743.2); PKD1 (L33243.1); PKD2 (NM_000297.2)

Table 2: Overview of the number of hepatic cyst tissues per PLD patient for evaluation of somatic hits. An extended number of hepatic cysts in several patients have been analyzed in PCLD compared to ADPKD; n=71 cysts and n=52 cysts in *PRKCSH* and *SEC63* affected individuals respectively.

Trans-heterozygous Model

The first hypothesis of a trans-heterozygous model was confirmed with experiments in renal cysts in a PKD1 affected ADPKD family. This study revealed that PKD1 germline derived cysts could have somatic PKD2 mutations [27]. Next, clonal PKD1 somatic hits were discovered in renal cysts from PKD2 carrier [31]. These observations demonstrate an alternative pathogenic mechanism for cyst formation. To date, the trans-heterozygous model has been excluded in PCLD. Co-existence of second hits at the PKD1 or PKD2 locus in PCLD may compromise the functional network in PLD [39,40].

Clinical Heterogeneity in Polycystic Liver Disease

The liver phenotype of inherited PLD may range from a single or few cysts to an advanced polycystic liver with numerous cysts. Usually, several liver segments remain unaffected and the liver function is preserved, even in severe PLD [2,3].

Individual and inter-patient differences of cyst size, localization and growth suggest that other factors are involved in the pathogenesis. The type of somatic mutations may explain to some extent the individual variability. For example, the number and type of somatic mutation in the tumor suppressor gene APC has important implications on the protein function resulting in growth advantages of the cell [41].

As indicated, the diversity of clinical presentation in families is high. The penetration of the disease has been estimated at ~80% and even if the disease become penetrant, its variability is high [10]. Some affected family members may develop early and severe disease, while others only develop minor symptoms. This may occur among those sharing identical germline mutations. Although family history is frequently negative in PLD, an asymptomatic carrier can transmit the disease to offspring who may become affected.

PLD patients may be asymptomatic for many years. The number and size of hepatic cysts increase progressively by the age of 30 resulting in advanced disease in patients in their fifth decade [4]. This variation reminds us of the situation in retinoblastoma. An early-onset of disease could be explained by the high rate of a second hits in embryonic cells [19]. The mutation rate of the somatic hit determines the progression of the disease. It is likely that in PLD these somatic hits are the rate-limiting step for PLD development.

Disease Model

Examination of the second hit model has afforded us insight in the molecular mechanisms of PLD. Genome-wide copy number and (copy number neutral) LOH regions from liver cyst tissues are of high interest, because these investigations may guide us to putative candidate genes for hepatic cyst formation. Next, LOH regions in cysts of target tissue (liver or kidney) may indicate modifiers or novel genes at the germline level which contribute to monoclonal hyperproliferation of cholangiocytes in congenital and sporadic cysts.

It is likely that more disrupted gene products are involved in PLD, because multiple pathways and modifiers affecting cystogenesis are identified. These reasons might explain similarities and differences in clinical presentation. For example, the polycystins are functionally part of similar signaling pathways. A recent study in animal models provided functional evidence for presence of trans-heterozygous mechanisms in PLD. Reduced hepatocystin or *Sec63p* expression leads to hepatic and renal cystogenesis, but also affects the functional polycystin complex. These data provide evidence for a PLD protein network responsible for

cystogenesis. Perturbation of polycystin-1 levels regulate the disease severity in a dosage-related fashion [39].

Concluding Remarks

The nature of individual cyst formation has been associated with genetic mechanisms that are common in tumorigenesis. For initiation and promotion of a malignant or benign tumor, more than 1 mutation is required [6,19]. In case of a hepatic cyst, both alleles of PLD-related genes are inactivated by a two-step process. The first hit is the inherited mutation present in the germline and the second hit is the onset of somatic events. Somatic hits can cover the spectrum from missense mutations to LOH. A fine example is the situation in liver cyst tissue from *PRKCSH* mutation carriers with a high LOH rate of 76% [34].

There is a high variability of liver phenotype in PLD patients that are of similar sex, age and share identical germline mutations. The focal character of intra-familial variability in PLD may be explained at least partially by genetic mechanisms at the somatic level. This paper lists the evidence supporting that inactivation of 2 PLD-gene copies underlies hepatic cyst formation.

Development of multiple fluid-filled cysts expands over time and affects the normal biliary architecture. These processes are influenced by age, gender (hormones), somatic hit rate and frequency, but probably also a significant dosage effect through accumulation of multiple mutations [18,39,40].

The genetic threshold is low in inherited PLD because there exists already loss of 1 allele. On the other hand, somatic inactivation of 2 PLD alleles is also possible and in those cases isolated cysts arise. Both modes result in clonally proliferation, growth and individual cyst formation.

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Author Contributions

WC and JD have contributed to the design, organization and writing of the review article. Authors declare no conflicts of interest.

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