Pathogenesis of Choledochal Cyst

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Contributor: Vincent Chi Hang Lui, Yongqin Ye, Paul Kwong Hang Tam

Choledochal cysts (CC) is characterized by extra- and/or intra-hepatic b\ile duct dilations. There are two main theories, “pancreaticobiliary maljunction” and “congenital stenosis of bile ducts” proposed for the pathogenesis of CC. Although family cases or CC associated with other anomalies have been reported, the molecular pathogenesis of CC is still poorly understood. Advances in transcriptomics and genomics analysis platforms have unveiled key expression signatures/genes/signaling pathways in the pathogenesis of human diseases including CC.

Keywords: choledochal cyst ; pathogenesis ; genomics ; transcriptomics

1. Introduction

Choledochal cysts (CC), also known as congenital biliary dilation, presents as the dilation of extrahepatic bile ducts. It has a worldwide incidence of about 1:100,000 to 1:150,000 but the incidence can be as high as 1 in 1000 in Asians. In addition, the disease incidence shows a female predominance, with a female to male ratio of 3~4:1. CC is often seen in infants, and can be diagnosed incidentally or when it causes biliary pancreatitis with abdominal pain, jaundice, and even liver enlargement. According to Todani’s classification [1], CC can be anatomically classified into 5 major types (type I–V). Type I, a dilation of extrahepatic common biliary duct (CBD), and type IV, a dilation of both intrahepatic bile duct and extrahepatic CBD, are the two most common types, accounting for about 50–80% and 15–35% of all the CC cases, respectively. Type II, a diverticulum of CBD, and Type III, a dilation of the duodenal portion of the CBD that bulges into duodenal lumen, are rarely seen in infants. Type V, also called Caroli disease, is characterized by multiple dilations of the intrahepatic bile ducts, and the disease is always accompanied with polycystic kidney disease (PKD) or congenital liver fibrosis (Caroli syndrome).

The etiology of CC remains unclear. There are two main theories, “pancreaticobiliary maljunction” and “congenital stenosis of bile ducts”, proposed for the pathogenesis of CC [2][3][4][5]. Pancreaticobiliary maljunction is a congenital malformation in which the pancreatic and bile ducts join outside the duodenal wall, forming an abnormally long common channel. In patients with pancreaticobiliary maljunction, the sphincter of Oddi fails to regulate the function of the pancreaticobiliary junction, resulting in a two-way regurgitation and a mixing of pancreatic juice and bile, which activates the pancreatic enzymes in the pancreatic juice, leading to bile duct damages and inflammation, which subsequently result in a dilation of the bile duct as seen in CC. However, pancreaticobiliary maljunction can only be identified in about 50–80% of patients with CC [6]. Congenital stenosis of bile ducts is characterized by a reduced number of ganglions and neurons of the CBD, which causes a dysregulated contraction and increased intralumenal pressure in the proximal bile duct, leading to a cystic distention of the proximal CBD in CC [7][8][9][10].

2. Chromosomal Anomalies

Chromosomal anomalies have been identified in CC (**Table 1**). Chromosomal duplications such as 17q12 are seen in type Ia CC [11]. 17q12 chromosomal region contains the *HNF1B* gene, and *HNF1B* mutations or 17q12 microdeletions are usually seen in patients with nephritic cysts, diabetes syndrome, and neurologic and psychiatric symptoms [12][13][14][15][16][17]. *HNF1B* encodes a transcription factor called hepatic nuclear factor-1 β (HNF1B), which is involved in bile duct organogenesis [18], and its deletion has been shown to induce jaundice, and anomalies of gall bladder [19]. Kettunen et al. evaluated 14 patients with *HNF1B* mutations and found 6 patients had CC, which exceeds the overall incidence of CC in the general population, indicating that mutations *HNF1B* in 17q12 may play a role in the pathogenesis of CC [20]. Later, Kotalova et al. reported that a two-week-old boy carrying a 17q12 duplication spanning 1698 kb that contains the *HNF1B* gene also suffered from CC, which is similar to the phenotype as seen in patients with a 17q12 microdeletion. The 17q12 duplication led to an overdosage of *HNF1B* and was found both in the young boy and his mother, indicating that the autosomal dominant inherence may exist, although his mother was completely within normal range [11].

Table 1. Chromosomal alterations reported in choledochal cyst patients.

Chromosomal Anomaly	Localization	Phenotype Description	Type	Candidate Gene	References
Duplication	17q12	Diabetes, renal disorders, structural brain anomalies, etc.	Ia	*HNF1B*	[11]
Deletion	Microdeletion 17q12	Diabetes, renal cysts, neurological and psychological anomalies, biliary or hepatic clinical phenotype	I, IV	*HNF1B*	[20]
Deletion	2p15p16.1	facial features, developmental delay Congenital microcephalyand intractable myoclonic epilepsy	II	*EHBP1*	[21]
Deletion	5q	Familial adenomatous polyposis	V	-	[22]
Translocation	t(3;8)(p23;q13)	Abdominal pain, jaundice	V	*APC*	[23]

De novo 2p15p16.1 microdeletion encompassing the *CCT4*, *COMMD1*, *B3GNT2*, and *EHBP1* genes has been shown to associate with multiple congenital anomalies including renal anomalies, intractable seizures, and a choledochal cyst [21][24][25][26][27][28][29]. Among these genes, *EHBP1*, the gene-encoding and actin-binding protein for cytophagy, was the only gene deleted in a type II CC patient [21]. Chromosome arm 5q deletion has been widely reported in familial adenomatous polyposis (FAP), which is due to mutations of the *APC* gene [30][31]. Deletion of chromosome 5q was once reported in a type V CC (Caroli disease) patient with FAP [22]. A cytogenetic study of a patient with a familial isolated Caroli disease revealed an unbalanced translocation between chromosome 3 and chromosome 8 with t(3;8) [23]. Genes located in 3p23 and 8q13 were rearranged, indicating that the distal loss and/or gain of 8q may contribute to the pathogenesis of Caroli disease. Genomic gains of 8q can promote ribosome biogenesis activity, which promotes hepatocellular carcinoma development [32][33]. CC has a higher risk of malignancy transformation [34][35][36], although a link between chromosomal translocation t(3;8) leading to a gain of 8q13-qter and the risk of malignancy transformation in CC has yet to be established.

3. Transcriptomics Analysis

There have been few microarray or RNA sequencing studies on the etiology of CC. Lv et al. performed transcriptome sequencing analysis on subtypes of Type I CC including cystic CC (Ia) and fusiform CC (Ic), and identified 6463 differentially expressed genes (DEGs) implicated in the biological processes of epithelial cell differentiation or extracellular matrix between these two CC subtypes [37]. Pathways related to metabolism and hormone regulation were enriched in cystic forms, while pathways related to immune response were enriched in fusiform CC [38][39]. An enrichment of immune-related genes is in line with a common clinical manifestation of immune-related complications including cholangitis and pancreatitis in fusiform CC [38][39]. Weighted gene co-expression network analysis (WGCNA) enables identification of gene modules and key genes that were correlated and relevant to clinical traits [40][41]. Using WGCNA, 12 co-expression modules were constructed, and the blue module, comprising the second most genes, was identified to be strongly correlated with fusiform CC [37], indicating that genes within this module could be potential markers for subtypes of CC. To further investigate key candidate genes in this module, a protein–protein interaction (PPI) was performed and found that the blue module contained key genes enriched in the Wnt signaling pathway, and activation of the Wnt pathway was associated with cholangio-carcinogenesis [42][43]. CC has a higher risk of malignancy transformation; whether an activation of Wnt pathway is involved in malignancy transformation of CC requires further investigation. *ERBB2* and *WNT11* were the two hub genes in this same module that distinguished fusiform CC and cystic CC. *ERBB2* mutations/amplifications were implicated in extrahepatic cholangiocarcinoma [44][45][46]. All of these indicated that the CC subtypes share some common transcriptomic signatures, but each one also displays its own unique transcriptomic signatures. Therefore, elucidation of their common and unique transcriptomic signatures for each CC subtype will not only provide insights into the molecular pathogenesis of each subtype but also help in clinical management of the disease. For example, if the subtype's unique transcriptomic signature suggests an elevated risk of cancer development, a more frequent follow-up and closer monitoring of any pre-malignant changes in the liver of post-surgical patients will help to identify patients for early cancer treatment.

4. Genetic Variants

A trio-based whole exome-sequencing of 31 CC trios was performed to identify de novo variants [47]. A total of 27 non-synonymous de novo variants were identified, 21 of which were damaging de novo variants, including 4 protein truncating mutations and 17 missense mutations. The constraint scores on average are statistically higher than random samples, indicating that these sequence variations are likely to contribute to CC. Six genes

(*PXDN*, *RTEL1*, *ANKRD11*, *MAP2K1*, *CYLD*, and *ACAN*) with the de novo variants are involved in human developmental diseases, such as sclerocornea, spondyloepimetaphyseal dysplasia, dyskeratosis congenita, and familial multiple trichoepitheliomata [48][49][50][51][52][53][54][55][56]. Furthermore, four genes (*PIK3CA*, *TLN1 CYLD*, and *MAP2K1*) with the damaging variants are linked to bile duct and liver cancer. De novo variants in significantly mutated regions (SMRs) genes (*PIK3CA*, *C6*, and *PPP2R2B*) were over-represented in CC patients, especially *PIK3CA* with excess mutations implicated in 9 cancer types, indicating that it may play a role in malignancy [57][58][59][60][61]. A total of 12 genes (*DCHS1*, *EPS15*, *DNM1*, *C5orf42*, *POU2F2*, *THBS1*, *POU2F2*, *BYSL*, *C5orf42*, *THBS1*, *BYSL*, *TXLNB*) with de novo damaging variants recurrently identified in different individuals, forming either protein–protein interactions (PPIs) or compound heterozygotes or in homozygous. PPI pair TRIM28 and ZNF382 were overrepresented in CC group compared to controls in PPI analyses, with 3 and 2 patients carrying rare damaging variants on these two genes, respectively. TRIM28, known for regulation of the endoderm differentiation and involvement in malignancy, may play a role in CC [62][63][64][65]. Though the whole exome sequencing study was limited by a small sample size, CC is regarded as a multigenetic disease with mutations in more than one gene with genetic heterogeneity [47].

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