Special article

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Genetic cholestasis: classification according to the cellular defect

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ABSTRACT

Advances in molecular biology achieved during the last years have allowed us to know the genes involved in biliary secretion and the mutations capable of generating cholestasis. The mechanisms involved in forming bile and its circulation have been clarified. According to the biology of biliary secretion, we classify the genetic causes of cholestasis as follows: 1) transport abnormalities in canalicular or basolateral membranes, 2) alterations in intracellular vesicle transit, 3) increased paracellular permeability, 4) mutations in nuclear receptors, 5) cholangiopathies, and 6) hepatocellular diseases, due to disturbance of the function of intracellular organelles or errors of metabolism.

This physiopathological classification of chronic cholestasis in childhood will facilitate pediatricians' diagnostic guidance and timely specialized referrals, as patients should receive early and appropriate treatment for its complications.

Keywords: *cholestasis; genetics; bile; genetic testing; therapeutics.*

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INTRODUCTION

Progress in molecular biology has led to the discovery of genes involved in biliary secretion and the mutations in these genes that cause cholestasis. Advances in cell biology offer the possibility of explaining the mechanisms by which the proteins encoded by these genes can modify or alter the secretion of bile towards the apical pole—that is, the biliary canaliculus of the hepatocytes—or disturb its transit towards the intestine through the biliary ducts.

The personal and family history of patients with cholestasis, physical examination, laboratory tests, and, in some cases, radiological studies, guide the search for a diagnosis that explains the clinical presentation. The accurate diagnosis will be obtained through genetic studies, although, for the moment, these are economically onerous and not universally accessible. Moreover, in some cases, they are not conclusive. Treatments to facilitate biliary secretion or protect liver cells from retaining highly detergent bile salts have been available for years. Malabsorption of fats and fat-soluble vitamins due to the decrease of bile salts in the lumen of the intestine imposes dietary modifications that must be promptly instituted. In very few cases, as in inherited errors of bile acid metabolism, a specific therapy with the administration of bile acids is available.

In some cases of hereditary cholestasis, these may be transient and without progression to hepatic parenchymal destruction; they are called "benign recurrent cholestasis".[1,2]

In general, these cases are caused by mild or heterozygous mutations, with preservation of partial function of the mutated protein, encoded by genes known to be responsible for progressive cholestasis in cases of severe mutations in the two alleles (autosomal recessive diseases).

PHYSIOPATHOLOGY

Decreased bile flow is a frequent anomaly in pediatric patients; the causes of this pathology are frequently of genetic origin. Biliary secretion begins in the hepatocyte; bile components are produced or imported by these cells and transported into the biliary canaliculus. Some of these components, such as bile acids, bilirubin, and cholesterol, recirculate from the intestine, are taken up by hepatocytes, and again secreted into the bile.[3]

Hepatocytes are related by intercellular junctions, among which tight junctions (*zonula occludens*) are responsible for the division of the plasma membrane of these cells into two domains with different functions: 1) the apical pole or biliary canaliculus, and 2) the basolateral pole, in contact with the circulating blood through the hepatic sinusoids. These cellular domains contain proteins and conform transporters and receptors with different functions. Mutations in the genes encoding these proteins modify the constitution of the bile and, consequently, produce cholestasis due to the retention of products toxic to the hepatocytes or due to the absence of products essential for the formation of normal bile.

Proteins manufactured in the endoplasmic reticulum are processed and transported by specialized vesicles to their destination, either the apical or basolateral pole. These vesicles circulate using the cytoskeleton as a pathway and are bound to it by a protein complex. Mutations of the genes encoding these proteins modify the circulation of vesicles, decreasing the number of transporters in the plasma membrane and, consequently, altering biliary secretion.

Alterations in DNA transcription and their consequence in the generation of mRNA result in the absence or decrease of a protein. Different nuclear receptors influence gene transcription. Mutations in the genes encoding some of the nuclear receptors generate a reduction of these receptors and, consequently, the failure in the transcription of genes encoding proteins involved in biliary secretion.

The functional alteration of hepatocytes due to the accumulation of toxic products in some metabolic diseases or a deficiency in the production of energy necessary in most metabolic processes can indirectly affect biliary secretion. Errors in the metabolism of bile acids modify bile formation, decreasing the available amount of an essential component, bile salts, and accumulating toxic products in the cell, which are the intermediates of bile acid metabolism.

Bile circulates from the bile canaliculi to the interlobular ducts. These and the larger ducts are lined by epithelial cells called cholangiocytes. Alterations in the embryological development of the bile ducts, in the function of the cholangiocytes, or the destruction of these by a relative increase in the concentration of bile salts in the bile produce bile retention and the consequent symptoms and signs of cholestasis.

PATHOLOGIES ACCOUNTABLE FOR CHOLESTASIS

According to the biology of biliary secretion,

we can classify the causes of cholestasis as those that are produced by:

1. Transport abnormalities in the canalicular or basolateral membranes.
2. Alterations of intracellular vesicle transit.
3. Increased paracellular permeability.
4. Mutations in nuclear receptors.
5. Cholangiopathies.
6. Hepatocellular diseases due to disturbance in the function of intracellular organilles or metabolic errors.

The diseases corresponding to each of these anomalies are described in *Table 1* and *Figure 1*.

Clinically, we can distinguish diseases in which cholestasis is the only or predominant manifestation from those in which cholestasis is part of a more complex syndrome.

According to laboratory tests, we can consider cholestasis with normal serum gamma-glutamyltransferase (GGT) and those in which GGT is increased.

Depending on the etiology, the liver function can be normal or insufficient at disease presentation.

DISCUSSION

The incidence of each of these diseases is relatively rare when analyzed individually. However, considering the genetic causes of cholestasis, they represent more than one-third of the etiologies during the first three months of life. Although the development of molecular biology techniques has made it possible to specify the cause in most cases, there are still patients without a definitive diagnosis.

Techniques for genomic diagnostics have advanced rapidly over the last two decades, resulting in broader availability and lower cost. The field of molecular diagnostics was revolutionized with the discovery of next-generation sequencing (NGS), which allows the simultaneous analysis of multiple genes. These techniques can be applied to specific genes associated with a particular clinical presentation (targeted gene panel), whole exome sequencing (WES), or whole genome sequencing (WGS). According to the patient's clinical presentation, it is advisable to start the genetic evaluation with a targeted gene panel and later extend it to the other two techniques if the diagnosis is inconclusive.

The greater accessibility of these techniques has allowed the identification of new genetic variants associated with neonatal cholestasis during the last decade (*Table 1*).

In a multicenter study conducted by researchers who developed one of the first panels to detect gene mutations related to cholestasis, results were reported on 66 genes in 2,433 patients without etiology. The list of genes examined in the search for mutations included those responsible for the formation and metabolism of bile acids, those responsible for peroxisomal or mitochondrial diseases, those accountable for cholangiopathies, those that modify paracellular permeability, or alter the transport of vesicles or molecules into the bile, and those that regulate the transcription of genes involved in biliary secretion. An essential aspect of this study is showing us the frequency of hereditary pathologies responsible for cholestasis. Alagille syndrome, a consequence of mutations in the *JAG1/NOTCH2* genes, is the most frequent disease, followed by *CFTR* deficiency, alpha-1-antitrypsin deficiency, and mutations in the *ABCB11* gene coding for the bile acid transporter in the membrane of the biliary canaliculus. Mitochondrial DNA polymerase gene (*POLG*) mutations are the most frequent cellular organelle pathology. Of the DNA analyzed, 265 (12%) showed mutations that allowed a diagnosis of inherited disease to be confirmed.[4]

Establishing a classification of the causes of hereditary cholestasis according to the mechanism or cellular or tissue structure affected by the genetic mutation makes it possible to imagine and design different therapeutic strategies.[5,6] The reproduction of mutations in cell culture models or laboratory animals facilitates the testing of different molecules and, in some cases, the implementation of gene therapy.[7,8]

Understanding the pathophysiological mechanisms of a disease allows a better interpretation of symptoms and signs, as well as the choice and follow-up of treatment. Strong diagnostic guidance is possible during the initial clinical and laboratory evaluation, limiting the excessive use of resources.

In conclusion, we propose a pathophysiologic classification of childhood chronic cholestasis to facilitate pediatricians' diagnostic orientation and timely referral to the specialist. There is no doubt that knowledge of the patient's genotype will allow us to establish a prognosis and to assess the need for and timing of liver transplantation.[9] ■

Table 1. Genetic variants associated with cholestasis in childhood

Location	Gene	Protein	Function	Symptoms and signs
a) Abnormalities in the transport of bile components in the apical and basolateral poles				
Canaliculus	*ATP8B1*	FIC1	Translocase of amino phospholipids	Neonatal cholestasis Diarrhea
	ABCB11	BSEP	Transport of bile salts	Neonatal cholestasis
	ABCB4	MDR3	Transport of phospholipids	Neonatal cholestasis
	ABCC2	MRP2	Transport of bilirubin bi-glucuronate	Increased serum direct bilirubin Dubin-Johnson syndrome
Basolateral	*SLC10A1*	NTCP	Import of bile acids	Cholestasis
	SLC01B1	OATP1B1 y 1B3	Import of bilirubin bi-glucuronate	Increased serum direct bilirubin Rotor syndrome
	SLC51A-SLC51B	OSTa-OSTb	Organic solutions	Cholestasis Diarrhea
b) Abnormalities of the intracellular transit of vesicles				
Vesicles	*VIPAS39*	SPE39	Cell polarity Vesicle recycling	Arthrogryposis, renal dysfunction, cholestasis
	VPS33B	VPS33B		
	VIPAR	VIPAR		
	VPS50	Subunit of the complex EARP/GARP11		
	MYO5B	Myosin Vb	Cell polarity Vesicle recycling	Cholestasis, congenital diarrhea
	UNC45A	Unc45A associated to myosin	Protein complex with myosin	Cholestasis, congenital diarrhea, bone fragility, deafness
c) Increased paracellular permeability				
Tight junctions	*CLDN1*	Claudin-1	Tight junction protein complex	Neonatal sclerosing cholangitis
	TJP2	Protein 2 of tight junctions	Tight junction protein complex	Neonatal cholestasis
	USP53	Specific ubiquitin 53	Interacts with TJP2	Neonatal cholestasis
	LSR	Lipoprotein receptor stimulated by lipolysis	Tight junction formation	Neonatal cholestasis
	PLEC	Plectin	Microtubules, microfilaments and intermediary filaments association with tight junctions	Neonatal cholestasis
d) Defects in the production of nuclear receptors				
Core	*NR1H4*	Farsenoid X receptor	Nuclear bile acid receptor Increases BSPE expression	Neonatal cholestasis
e) Cholangiopathies with bile duct paucity				
Associated with microtubules	*KIF12**	Member 12 of the kinesin's family	Development of bile ducts	Cholestasis Bile duct paucity
Core	*HNF1B**	HNF1 "homebox B"	Nephron development	Cholestasis. Bile duct paucity Renal cysts
Plasma membrane	*ABCC12*	ATP - "binding cassette", subfamily C, member 12	Molecular transport	Cholestasis Bile duct paucity
Plasma membrane	*JAG1-NOTCH2*	Jagged 1 and its Notch2 receptor	Vascular development	Alagille syndrome Bile duct paucity

**Associated with microtubules and ciliary function.*

f) Cholangiopathies secondary to ciliary anomalies

Location	Gen	Protein	Function	Symptoms and signs
Cytosol-tubulin	*DCDC2*	Double-tethered curtain Z domain	Increases microtubule polymerization	Cholestasis Neonatal sclerosing cholangitis
Centrosomes	*ZFYVE19*	"Zinc fineger" FYVE19	Affects ciliary function	Neonatal cholestasis
Cilia-centrosomes	*INVS*	Inversina	Tubular (renal) development	Cholestasis
	NEK8	NIMA related kinase 8		Nephronophthisis
	NPHP9	Nephrocystin		
Cytosol	*CC2D2A*	"Coled-coil"C2, A2 domain	Alteration of cilia formation	Cholestasis
Basal body of cilia	*MKS1* *TMEM216*	MKS complex Transmembrane protein 216	Alteration of cilia formation	Cholestasis
Core	*HNF1*	HNF1 »homeobox B»	Nephron development	Cholestasis. Bile duct paucity
Plasma membrane	*PKD1L1*	Polycystin 1	Ciliary function	Cyst formation

g) Transport in cholangiocytes

Location	Gen	Protein	Function	Symptoms and signs
Plasma membrane	*CFTR*	Chlorine channel	Chlorine excretion	Cystic fibrosis

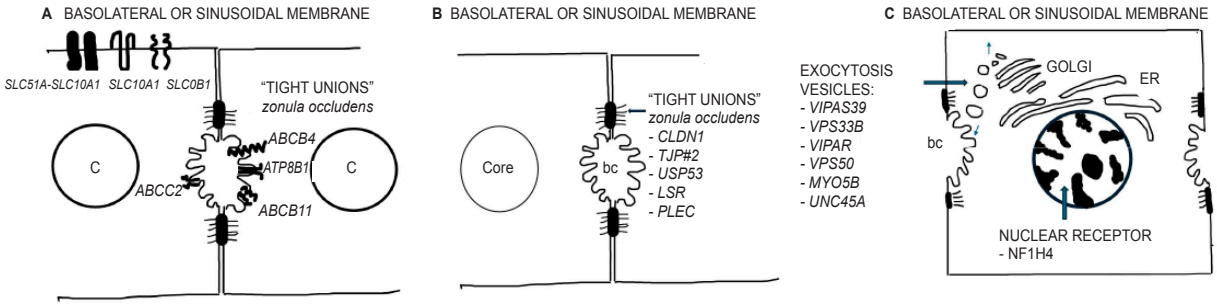
h) Disturbance of the hepatocellular function

Location	Gen	Protein	Function	Symptoms and signs
Endoplasmic reticulum	*SERPINA*	Alpha-1-antitrypsin	Anti elastase	Cholestasis
Cytosol	*GALT*	Galactose-1-phosphate uridyltransferase	Galactose metabolism Liver failure	Cholestasis
Mitochondria	*CYP27A1*	27-hydroxylase	Bile acid metabolism	Cholestasis
Peroxisomes	*EHHADH*	Enoyl-CoA hydratase 3-hydroaxyl CoA dehydrogenase	Oxidation in peroxisomes	Cholestasis Peroxisome disease

i) Errors of bile acid metabolism (enzymes most frequently involved)

Location	Gen	Protein	Function	Symptoms and signs
Endoplasmic reticulum	*HSD3B7*	3B-hydroxy-C27-steroid oxidoreductase	Bile acid metabolism	Cholestasis
Cytosol	*AKR1D1*	4-3-oxoesteroid-5B reductase	Bile acid metabolism	Cholestasis
	BAAT	Amidation of amino acids N-acetyltransferase	Bile acid amidation	Cholestasis

Figure 1. Different mechanisms of biliary excretion are modified by mutations in particular genes that produce chronic cholestasis in childhood



A. Transporters in biliary canaliculus and sinusoidal membranes affected in chronic cholestasis. B. Mutated genes that facilitate increased paracellular permeability. C. Mutations in genes that disrupt vesicle transit from the Golgi apparatus to cell membranes. ER: endoplasmic reticulum; C: core; bc: bile canaliculi.

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