

## GENETIC ASPECTS OF BILIARY ATRESIA ETIOLOGY

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Biliary atresia (BA) is a cholestatic disorder of infancy that is fatal if untreated. Despite years of study the etiology of BA remains unknown. Three etiopathogenic mechanisms may be involved, such as immune dysregulation, environmental factors and genetic susceptibility. Genetic predisposition is being actively studied. Candidate genes associated with BA in certain populations, genes affecting the cholangiocyte cilia function, as well as genes involved in stress responses have been identified. However, the long-term follow-up of twins with BA suggests that genotype is not of paramount importance for the disease development. Both epigenetic patterns and postzygotic somatic mutations may contribute to etiology of the disease. Recently, some evidence is being accumulated on the possible genetic predisposition to certain outcome of Kasai portoenterostomy performed in patients with BA. However, the presence of a number of factors contributing to the development of the disease makes it difficult to identify the genetic markers.

**Keywords:** biliary atresia, biliary atresia etiology, cholestasis, liver disease, genetic factors

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## ГЕНЕТИЧЕСКИЕ АСПЕКТЫ ЭТИОЛОГИИ БИЛИАРНОЙ АТРЕЗИИ

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Билиарная атрезия (БА) — это холестатическое заболевание печени, которое дебютирует в младенческом возрасте и при отсутствии своевременного лечения приводит к циррозу печени с прогрессирующей печеночной недостаточностью. Несмотря на многолетние исследования, этиология билиарной атрезии остается не до конца изученной. Среди факторов, вовлеченных в этиопатогенез БА, выделяют иммунную дисрегуляцию, факторы окружающей среды и генетическую предрасположенность. Роль генотипа пациента в развитии заболевания активно изучается, выявлены гены-кандидаты, ассоциированные с БА в определенных популяциях, гены, влияющие на функционирование ресничек холангиоцитов, а также гены, участвующие в стресс-реакциях. Однако, исходя из многолетних результатов наблюдений близнецов с БА, можно сделать вывод о том, что роль генотипа не является первостепенной в развитии заболевания. Предположительно, в этиологию заболевания могут вносить вклад как эпигенетические механизмы, так и постзиготические соматические мутации. В последнее время накапливаются также данные о возможной генетической предрасположенности к исходу портоэнтеростомии по Касаи, проводимой при БА. Вместе с тем, наличие многих факторов, играющих роль в развитии заболевания, создает трудности в выявлении точных генетических маркеров.

**Ключевые слова:** билиарная атрезия, этиология билиарной атрезии, холестаза, заболевание печени, генетические факторы

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Biliary atresia (BA) is an inflammatory fibrosing obliteration of the extrahepatic biliary tree gradually involving the intrahepatic biliary system and leading to cirrhosis. The isolated (nonsyndromic) forms of the disease are more common (85% of cases) compared to syndromic (embryonal, 10–15% of cases) and cystic forms (5–8% of cases). The incidence of BA varies across countries from 1 : 8,000 (Asia and Africa) to 1 : 18,000 (Europe), and more common for female infants [1, 2]. The clinical presentation of the disease is neonatal cholestasis.

However, the differential diagnosis is carried out with a large group of congenital and hereditary disorders, many of which have manifestations similar to BA during the first months of life [3, 4]. The diagnosis is confirmed by morphological examination of liver and bile duct biopsies during surgery. Surgical correction (Kasai portoenterostomy, KPE) and liver transplantation make it possible the overall infants survival with BA to 90% [4–6]. However, the causes of BA, as well as factors determining the treatment outcome remain poorly understood.

## Etiology of the disorder

Prior to becoming familiar with genetic patterns associated with BA and contributing to the treatment outcome, the other factors involved in the BA pathogenesis should be mentioned briefly: immune dysregulation and environmental factors (viruses, toxins).

There is a lot of data on etiological factors, among which the immune dysregulation plays an essential role. BA is a fibro-inflammatory disease characterized by presence of inflammatory cell infiltration and cytokines and/or chemokines overexpression in patients' liver biopsies. The key element in the BA immunopathogenesis is the innate immune response involving activation of NK cells and Th1 cells subpopulation, also known as Th1 adaptive immune response involving the effector T cells, which results in inflammation and obstruction [7]. At the same time, there is a decrease in the number of Treg cells, which are able to suppress inflammation. After the onset of obstruction the immune-mediated bile duct damage persists due to activation of Th2 and Th17 responses regardless of the bile outflow restoration [8]. However, no post-transplant disease recurrence is observed, in contrast to other immune-mediated bile duct diseases [9].

The viral or toxic effect on the bile duct epithelium is likely to give rise to new epitopes contributing to initiation and exacerbation of autoimmune inflammation [8, 10]. The following pathogenic viruses are considered: CMV, HBV, human herpesvirus 6, EBV, reovirus and rotavirus [11]. Numerous studies using the PCR technique (detection of viral DNA/RNA) or viral IgM<sup>+</sup> and Mx protein immunostaining revealed traces of viral infection in some, but not all, liver tissue specimen [12, 13]. Currently, there is no clear evidence of BA resulting from viral infection due to inconsistent results of studies using no reference samples, methodological inaccuracies and ambiguous data interpretation [10, 11, 14]. Paradoxically, BA does not affect adults infected with these viruses. But viral infection aggravates the course of BA and increases the risk of adverse outcome [12, 13, 15].

Of exogenous toxins able to trigger the outbreak of BA in animals, the previously undescribed isoflavonoid biliatresone was isolated in Australia from plants consumed by livestock during the drought [16]. In zebrafish (*Danio rerio*) larvae, being a common animal model, biliatresone induces the damage to extrahepatic, but not to intrahepatic bile ducts [17]. Despite the fact that humans are not exposed to biliatresone, recognition of bile ducts damage patterns may contribute to uncovering toxins capable of affecting humans.

## Genetic factors and BA

In contemporary literature more data on the patients' genetic susceptibility to BA becomes available. The inheritance of biliary atresia is not Mendelian. As a result, the disorder is not inherited, although several such cases have been reported [18]. High incidence of BA in parts of Asia suggests the greater distribution of genetic variants associated with BA in these populations. However, the environmental factors (nutritional status, viral load, etc.), as well as the differences in diagnostic criteria used by Asian specialists should also be considered [19].

## Twins with BA

Genes that are likely to be involved in biliary atresia are discussed below. The intriguing results of meta-analysis reported in 2020

are worth noting: the authors reviewed the previously reported global cases of twins with BA and discovered that among 35 prominent pairs (19 monozygotic, 15 dizygotic, and one undefined) only one pair (dizygotic) demonstrated concordance for BA, and the other pairs were discordant, i. e. only one twin out of two was diagnosed with BA (97.1%) [20]. A retrospective study performed by Chinese researchers also reported 19 twin sets with BA, all of them demonstrated discordance for BA (8 were monozygotic and 11 were dizygotic) [21].

The twins' discordance for BA suggests that hereditary factors are not of paramount importance for the disease development, since monozygotic twins possess the same genotype. Meanwhile, contamination by infectious or toxic agent should rather have affected both twins in utero leading to the development of the disease. Among all reported cases of toxic or infectious embryopathy, the twins' concordance (especially monozygotic) is about 80% [22]. It is known that in addition to gene nucleotide sequence alterations, the epigenetic modifications possessing nonclassic genetic inheritance pattern may affect the phenotype. Thus, the monozygotic discordant twins with BA had different phenotypes, which increased the likelihood of the epigenetic factors contribution to the BA pathogenesis [20].

The post zygotic somatic pathogenic variant may also occur in one of the twins, within genes, regulatory regions, etc. being the potential triggers of obliterative cholangiopathy. The hypothesis about BA resulting from somatic mutation (it is better to use the term "pathogenic variant") was proposed in 2016. The authors suggest to analyze DNA from liver tissue and biliary tract of the patients, as well as to perform analysis of parental genomes in order to justify the hypothesis [22].

## Candidate genes

Genetic approaches to BA include the analysis of candidate genes, CNV (copy number variation), genome-wide association studies (GWAS), and whole exome sequencing (WES). GWAS are focused on evaluation of associations between the disorder and the common genetic variants in various populations of the treatment group of patients and the control group of healthy individuals. The table presents some candidate genes associated with BA identified in a number of GWAS.

## Genes *GPC1* and *AGXT*

During one of the GWAS, in two of 35 unrelated children with verified BA (2,026 healthy individuals served as controls) the heterozygous deletion of 2q37.3 was identified [23]. The overlapping deletion (1.76 Mb) contained 30 genes, of which the authors selected the candidate gene *AGXT* expressed in liver. Alanine-glyoxylate aminotransferase (*AGXT*), the hepatic peroxisomal enzyme involved in metabolism of toxic substances and lipid cleavage, is encoded by this gene. It can be assumed that the reduced activity of *AGXT* involved in detoxication is essential under the context of the toxin-mediated damage to biliary tract involvement in the pathogenesis of BA. The authors presented a detailed description of patients carrying a deletion 2q37.3. In the first case, the woman worked for a house cleaning service and was often exposed to potentially toxic cleaning agents during pregnancy. In the second case, the woman had a varicella infection at 15 weeks gestation, and was treated with acyclovir, which crossed the placental barrier and was metabolized in hepatocytes. However, in one of cases the patient's father also carried the deletion 2q37.3, but had no liver disease. Therefore, the deletion 2q37.3 may be considered

**Table.** Candidate genes associated with BA according to GWAS

Locus	Ethnic group	Variant	Candidate gene	GWAS
2q37.3	Caucasian	Heterozygous deletion	<i>GPC1/AGXT</i>	23/24
14q21.3	Caucasian	Non-coding SNP	<i>ARF6</i>	25
10q24.2	Han Chinese	Non-coding SNP	<i>ADD3/XPNPEP1</i>	26/27/28
2p.16.1	Caucasian	Non-coding SNP	<i>EFEMP1</i>	29

**Note:** SNP — single nucleotide polymorphism.

a factor contributing to susceptibility to BA, possibly due to biotransformation of xenobiotics.

In the other study the same researchers increased the sample size to 61 patients with BA vs. 5,088 healthy controls [24]. Deletions 2q37.3 of various lengths were identified in six patients (9.84%) and four healthy individuals (0.08%). However the region of interest contained the deletion of one *GPC1* gene copy. The gene encodes glypican involved in regulation of Hedgehog signaling and inflammation. Knockdown of *gpc1* in zebrafish (*Danio rerio*) overactivated the Hedgehog signaling resulting in the developmental biliary defects, smaller gallbladder and poor bile excretion. In the specimens obtained from patients, reduced GPC1 staining was observed on the apical surface of cholangiocytes. The authors concluded that gene *GPC1* appeared to be a BA susceptibility gene.

The case-control study performed by Chinese researchers revealed a possible association with reduced BA risk in 50% of patients with the following *GPC1* haplotypes:  $C_{rs2292832} - C_{rs3828336} & T_{rs3828336}$  or  $T_{rs2292832} - T_{rs3828336}$  [30].

### Genes *ADD3* and *XPNPEP1*

During the very first GWAS carried out in Chinese population the 500,000 single-nucleotide polymorphisms (SNP) were genotyped in 200 patients with BA and 481 healthy controls [26]. The strongest overall association was found for rs17095355 located on 10q24.2 between genes *XPNPEP1* (X-prolyl aminopeptidase) and *ADD3* (adducin 3). However, the authors failed to determine how the intergenic variant could affect the susceptibility to BA, but suggested that regulation of neighboring genes was involved.

*ADD3* encodes adducin 3, the protein belonging to a family of membrane skeletal proteins involved in the spectrin-actin network assembly in erythrocytes, and at sites of cell-cell contact in epithelial tissues, including organs of the gastrointestinal tract, liver and biliary tract [31, 32]. The highest, compared to adults, expression of *ADD3* is observed in fetal hepatocytes and cells of biliary ducts [32]. The contraction of intrahepatic bile canaliculi facilitating the bile drainage is controlled by the actin-myosin interaction. It is important to note that during the experiment with medications the impaired interaction resulted in severe cholestasis [33]. Increased accumulation of actin and myosin around bile canaliculi was observed in patients with BA who did not exhibit bile flow after surgery [34]. Moreover, the  $\alpha$ -smooth-muscle actin expression intensity correlated with the degree of fibrosis in patients with BA [35].

*XPNPEP1* is expressed in the biliary epithelial cells [36], it encodes the soluble X-prolyl aminopeptidase or the soluble aminopeptidase P (APP1). The APP1 enzyme contributes to degradation of bradykinin (BK) and substance P (SP) [37]. Bradykinin is involved in vasodilation and vascular permeability, the expression of bradykinin is regulated directly by the nuclear bile acid receptor, farnesoid X receptor (FXR), which plays a part in regulation of bile acid synthesis and secretion, and is involved in inflammation [38–39]. The inflammatory mediator SP is also involved in regulation of bile secretion, hepatobiliary

transport and innervation of the liver. The role of hepatobiliary transporters (particularly the FXR) was studied in murine models [40].

This study attracted attention to locus 10q24.2. Several studies of the locus were carried out in different populations [41–46]. It's worth mentioning that the significant association between the described polymorphism rs17095355 and BA was also revealed in Thai population [42].

However, the study of the North American patients' cohort revealed no association to rs17095355, but revealed the association of BA and the other SNP (rs7099604), located in intron 1 of the *ADD3* gene [41]. The quantitative PCR detected significant differences in the expression of *ADD3*, not *XPNPEP1*, in liver tissue between diseased and healthy individuals, but there were no differences in the *ADD3* nucleotide sequence between the groups. Therefore, the diseased individuals may have alterations in the non-coding regulatory DNA regions or epigenetic modifications.

In 2020, the association of three SNPs in *ADD3* (rs17095355, rs10509906 and rs2501577) and two SNPs in *GPC1* (rs6750380 and rs6707262) with BA in the group of Chinese patients ( $n = 340$ ) was validated [59].

The first model of BA using the induced pluripotent stem cells (iPSCs) was developed in 2019 [60]. The iPSCs obtained from the BA patients' blood differentiated *in vitro* into pathological cholangiocytes with signs of fibrosis. The researchers integrated the BA-associated SNP in *GPC1* and *ADD3* in healthy iPSCs using the CRISPR/Cas9 system and induced the biliary differentiation. These cells reproduced the pathological development of cholangiocytes as BA-specific iPSCs. The iPSC-based models of BA hold great promise for further study of the BA pathogenesis.

### Gene *ARF6*

The BA-associated SNPs rs3126184 and rs10140366 in *ARF6* (ADP-ribosylation factor) located in 14q21.3 were identified in 2015 [25]. The minor alleles of these polymorphisms were associated with reduced expression of *ARF6*.

Genes *ARF6*, *GPC1* and *ADD3* have similar functions in the formation and development of the biliary tract. *GPC1* and *ARF6* are involved in FGF (fibroblast growth factor) and EGF (epidermal growth factor) signaling playing an important role in organogenesis. Along with *ADD3*, *ARF6* regulates the actin cytoskeleton remodeling, affects the cellular motility and intercellular junctions. *ARF6* is activated by binding to the EGF receptor (EGFR) with its activator GEP100, which in turn sequentially triggers other reactions. The further activation of EGFR-GEP100-*ARF6* results in activation of MAPK/ERK-CREB signaling cascade, which ultimately affects normal cell development and proliferation [49–52].

Currently, there is no single point of view whether changes in the intrahepatic bile ducts are secondary or independently formed. Thus, four infants diagnosed with BA during their first days of life underwent a number of biopsies, which revealed the paucity of intrahepatic bile ducts with no signs of fibrosis

and cirrhosis always found during Kasai portoenterostomy or liver transplantation [53]. As early as in 1974 Landing proposed that the entire biliary tree was involved in obstructive cholangiopathies: the involvement of intra- and extrahepatic bile ducts hinged of the duration and type of noxi-influence [54].

The *arf6* knockdown in zebrafish (*Danio rerio*) larvae resulted in reduced liver size, lower number of biliary epithelial cells, poor bile excretion and impaired development of extra- and intrahepatic bile ducts. Similar effects were observed upon injection of EGFR inhibitor. The authors note that the EGFR-Arf6 signaling pathway may contribute to the intrahepatic bile ducts morphogenesis [25].

Thus, in two of 29 biopsy specimens taken from patients with BA the weak ARF6 immunostaining was detected, as well as the reduced number of intrahepatic bile ducts with signs of fibrosis. Based on these data the authors suggested that the *ARF6* expression downregulation facilitated the defective formation of both extra- and intrahepatic biliary network [25].

### Gene *EFEMP1*

The new susceptibility locus for BA in 2p.16.1 was revealed both in infants with isolated form of BA and with BA combined with other abnormalities [55]. The three BA-associated SNPs, rs10865291, rs6761893 and rs727878B, were detected within intron 5 of *EFEMP1* gene of the described locus. More *EFEMP1* transcripts in the liver tissue (mainly in cholangiocytes and portal fibroblasts) were observed in patients with either BA or other cholestatic diseases compared to control group.

*EFEMP1* gene encodes fibulin-3 involved in the extracellular matrix remodeling, tissue regeneration and organogenesis [56, 57]. Furthermore, *EFEMP1* is an activator of the Notch signaling *in vitro*, although is less efficient than *JAG1* [58]. It is known that fibulins interact closely with laminins and other extracellular proteins. Considering the close contact and proximity of the developing bile ducts with the portal mesenchymal tissue extracellular matrix, we can assume that the *EFEMP1* protein is involved in the biliary tract development.

However, the study of Chinese patients with BA performed in 2020 revealed no associations to genes *EFEMP1* and *ARF6* [59].

### Genes *STIP1* and *REV1*

In 2020, the trio analysis of exomes in 30 families with BA in children revealed 66 de novo variants in 66 genes including potentially deleterious variants in *STIP1* and *REV1* [61]. The proteins encoded by these genes interact with the heat-shock protein HSP90 and are involved in stress-responses. The other group of researchers introduced mutations to genes *stip1* and *rev1* of zebrafish (*Danio rerio*) using the CRISPR/Cas9 system and exposed the fish to biliatresone: in contrast to wild-type fish, the mutant fish were sensitive to low doses of biliatresone [62]. The *in vitro* knockdown of these genes in cholangiocytes and exposure to biliatresone disrupted the cytoskeleton. These results support the hypothesis of the environmental contribution to BA in people with genetic susceptibility.

### Genes of cholangiocyte cilia

The trio-WES of 89 families with BA in children was carried out in 2020 [63]. The researchers detected rare deleterious de novo variants in ciliary genes of 31.5% patients and identified three candidate genes: *KIF3B*, *PCNT* and *TTC17*. The patients carrying mutations in these genes had low levels of *KIF3B* and *TTC17* proteins in liver tissue. The knockout of *kif3b*, *pcnt* and

*ttc17* using the CRISPR/Cas9 system resulted in impaired bile outflow in zebrafish (*Danio rerio*) larvae. The researchers suggest that the defective cilia may cause hyperactivation of Hedgehog signaling. Abnormal cholangiocytes may also be damaged by bile, which facilitates Hh signaling and leads to inflammation and fibrosis.

### Genetic factors contributing to the outcome of BA

Specific genetic characteristic of the patient may significantly affect the disease severity. Despite the BA phenotypic heterogeneity, there are relatively few studies of genetic factors contributing to the surgical treatment (Kasai portoenterostomy) outcome. According to some authors, such genes as *A1AT*, *JAG1* and *CFTR* may contribute to surgical outcome [64–68].

The  $\alpha 1$  antitrypsin deficiency is a monogenic autosomal recessive disorder (*A1AT ZZ* genotype) leading to liver disease in children. It has been shown that pathological alleles like Z, S, etc. (heterozygous) are more common in children with chronic liver disease ( $n = 241$ ), including those with BA ( $n = 67$ ), than in general population [69]. The BA children with such genotypes had lower age of liver transplant compared to BA children with normal MM genotype.

The WES analysis of DNA extracted from liver biopsies of 20 patients with BA obtained during portoenterostomy was carried out in Thailand [70]. After surgery, only seven patients showed signs of jaundice clearance, three patients improved partially, and in 10 patients, surgery had no effect. The 13 rare variants in nine genes responsible for known diseases, including cholestatic disorders, were identified in BA patients (no clinical manifestations of these disorders were observed): *JAG1* (Alagille syndrome, AGS); *MYO5B* (congenital microvillus atrophy progressive familial intrahepatic cholestasis type 6); *ABCB11* (familial intrahepatic cholestasis type 2); *ABCC2* (Dubin–Johnson syndrome); *ERCC4* (Fanconi anemia); *KCNH1* (Zimmermann–Laband syndrome); *MLL2* (Kabuki syndrome); *RFX6* (Mitchell–Riley syndrome) and *UG1A1* (Crigler–Najjar syndrome type I). The authors suggest that BA and other liver diseases may have shared etiopathogenesis. Such associations are responsible for disease severity and poor prognosis in BA patients with native liver.

The missense *JAG1* mutations were identified in nine of 102 BA patients with no typical Alagille syndrome phenotype [71]. According to the authors, children with such variants had worse prognosis and portoenterostomy outcome. However, recent studies have shown that Alagille syndrome (AGS) may mimic BA: five children carrying the pathogenic variant in *JAG1* diagnosed with BA in early infancy have developed the typical for AGS clinical signs by 3 years, of age [72].

### Contribution of hepatocellular transporters and nuclear bile acid receptors to portoenterostomy outcome

Over the past decade the large amount of data was obtained describing the liver regeneration due to regulation of hepatocellular transporters (BSEP, MDR1, MDR3, OSTb) and nuclear bile acid receptors (FXR, PXR, CAR) activity in the context of cholestasis [73–75].

Liver adapts well to bile acids accumulation. It has been found that in healthy children the genetic deficiency of these receptors is of no clinical significance due to mechanisms of compensation. However, in patients with cholestatic disorders, including BA, the described alterations may become additional factors affecting the pathologic process. In case of hepatocellular transporters normal functioning

hepatocytes are protected from the toxic effect of bile acids due to their elimination by the BSEP transporter, and the biliary epithelium is protected due to major transporters FIC1 and MDR3 [76].

Based on these data, the studies has been carried out aimed at assessment of hepatic nuclear factors and hepatocellular transporters expression levels as predictors of KPE outcome in children with BA.

It was found that the expression of PXR and/or CAR receptor genes in the liver tissue of patients with poor KPE outcomes was significantly lower compared to patients with favourable outcomes. Five of six patients with low expression of both genes required liver transplant before one year of age (7–11 months) [75]. It had been previously shown that in Pxr knockout rats the liver damage caused by bile acids accumulation was significantly higher compared to healthy animals [77, 78]. It is believed that low levels of CAR and PXR may be associated both with genetic factors and inflammation. It has been determined that these nuclear bile acid receptors mediate the bile acid homeostasis by binding bile acids, are transported to nucleus and downregulate genes encoding the enzymes involved in the bile acids synthesis and reabsorption. At the same time they upregulate genes encoding transporters BSEP, MRP4 and OST $\alpha$ -OST $\beta$  responsible for export of bile acids from hepatocytes [79–90].

WES analysis was carried out aimed at searching for genetic variants more common in BA patients who required the liver transplant at early age due to no KPE effect than in children with native liver [91]. Among 98 children who required the liver transplant at early age the nonsynonymous variant p.A934T in *ABCB4* was more common compared to a group of children ( $n = 97$ ) who survived portoenterostomy with their native liver. Downregulation of *ABCB4* encoding MDR3 leads to the decrease in biliary phospholipids level, thus leading to bile acids damaging cholangiocytes.

The whole transcriptome mRNA sequencing of 29 liver samples obtained from patients with BA and differential expression analysis carried out in 2020 [92] identified the potential determinants of the KPE outcome: matrix metalloproteinase 7 (MMP7) and phosphoenolpyruvate carboxykinase (PCK1). MMP7 enzyme is involved in extracellular matrix remodeling in liver fibrosis; PCK1 is involved in gluconeogenesis, and its role in BA remains unclear. MMP7 expression was significantly elevated in patients who failed to clear jaundice after KPE as well as in patients with end stage liver disease. In contrast, PCK1 level was upregulated in patients who had successful KPE, while there was a significant downregulation in patients who failed KPE.

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Thus, the expression patterns of various genes in liver tissue and bile ducts may be used as biomarkers for prediction of KPE outcome allowing the healthcare specialists to develop new BA treatment strategies.

## Epigenetic factors

The BA pathogenesis may be based on epigenetic modifications (for example, DNA methylation, histone modifications, expression of non-coding RNA. etc.). DNA methylation was significantly reduced in bile duct cells from BA patients compared to patients with other cholestatic disorders [93]. That could lead to IFN $\gamma$ -signaling activation and inflammation. Various epigenetic modifications were identified in peripheral blood leukocytes (for example, CD4<sup>+</sup> T, Treg) of a number of BA patients [94–97].

It has been shown that hypomethylated *PDGFA* gene (platelet derived growth factor subunit A) is upregulated in the affected liver biopsy specimens possibly contributing to the BA pathogenesis [98]. The PDGF family proteins induce proliferative and fibrotic disorders in many organs. Thus, the variant rs9690350 (G>C) in *PDGFA* was associated with increased risk of BA in 506 BA patients when compared to 1473 healthy individuals [99].

The expression level of some miRNAs in the liver of BA patients was different compared to a group of healthy individuals. For example, mir-29b and mir-142-5p were overexpressed in the liver, despite the fact they targeted genes encoding key enzymes DNMT1 and DNMT3 involved in DNA methylation [100]. The other miRNA, mir-145-5p, targeted *ADD3* gene, it was downregulated in liver tissue of some BA patients [101].

## CONCLUSION

Despite years of study the etiology of BA remains poorly understood. Genetic research revealed no specific alterations. The disorder appears to have multifactorial etiology, which includes genetic alterations (inherited or somatic mutations), and epigenetic modifications due to genetic alterations or environmental factors (toxins, viruses).

The BA surgical correction (Kasai portoenterostomy) proposed as early as in 1955 makes it possible to preserve the liver function and to postpone transplantation. The factors affecting the surgical intervention effect and overall survival in patients with native liver remain understudied. Specific genetic characteristic of the patient as well as expression patterns of various genes in liver tissue and bile ducts are considered as prognostic biomarkers. However, further research is required.

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