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Environmental basis of primary biliary cholangitis

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Impact statement

Autoimmunity is believed to develop in genetically susceptible hosts with triggers from the environment. Researchers have recently demonstrated that bacteria and xenobiotics commonly present in our environment are potential triggers of tolerance breakdown against autoantigens and autoimmunity, particularly in primary biliary cholangitis (PBC). The link between xenobiotics and PBC has been further confirmed with the establishment of PBC model mice by immunizing mice with xenobiotics.

Abstract

Autoimmunity is a consequence of both genetic and environmental factors, occurring in genetically susceptible hosts with environmental triggers. While genome-wide association studies have revealed a number of susceptible genes contributing to etiology, the environmental triggers remain poorly understood. Primary biliary cholangitis, formally known as primary biliary cirrhosis, is considered a model autoimmune disease for which our group has extensively evaluated environmental factors involved in its etiology. Bacterial infection and xenobiotics have been proposed as candidate environmental factors that may explain tolerance breakdown and production of primary biliary cholangitis-specific antimitochondrial autoantibodies. Large-scale case-control studies have consistently detected an association of primary biliary cholangitis with urinary tract infections caused by *Escherichia coli*, as *E. coli* PDC-E2 is molecularly similar to human PDC-E2, the immunodominant target of AMAs. Another bacterium of interest is *Novosphingobium aromaticivorans*, a ubiquitous xenobiotic-metabolizing bacterium that produces lipoylated proteins, which are highly reactive with sera from primary biliary cholangitis patients. Regarding xenobiotics, case-control studies have suggested that frequent use of nail polish is associated with an increased susceptibility to primary biliary cholangitis. We found that 2-octynamide, the conjugate derived from 2-octynoic acid present in cosmetics, lipsticks, and some chewing gums, was unique in both its quantitative structure–activity relationship analysis and reactivity with primary biliary cholangitis sera. 2-nonyamide is another xenobiotic that also has the optimal chemical structure for xenobiotic modification of the PDC-E2 epitope, as demonstrated by the enhanced epitope recognition with AMA-positive PBC sera. Moreover, we found that C57BL/6 mice immunized with 2-octynoic acid-BSA possess many of the features characteristic to primary biliary cholangitis.

Keywords: Environment, primary biliary cholangitis, genetic background, anti-mitochondrial autoantibodies, animal models, autoimmune

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Introduction

Autoimmunity develops when immune responses target self-molecules due to breakdown of self-tolerance,[1] which can lead to cellular and tissue injury in various autoimmune diseases.[2] Accumulating evidence suggest that autoimmune reactions develop in genetically susceptible individuals with triggers from the environment.[3,4] Recent data from genome-wide association studies (GWAS) have revealed that the number of genes and pathways associated with the susceptibility or progression of autoimmune diseases has greatly increased over the years.[5] Due to the complexity of autoimmune diseases,[6] the environmental elements triggering their development remain poorly understood.[7]

Primary biliary cholangitis (PBC), formally known as primary biliary cirrhosis, is considered a model autoimmune disease because of its marked female predominance,[8] presence of disease-specific autoantibodies (i.e. AMAs), dense infiltration of mononuclear cells into bile ducts, and high prevalence of concomitant autoimmune diseases.[9] Like other autoimmune diseases, PBC is a multifactorial disease thought to be caused by interactions between

both genes and environmental triggers.[10] We have extensively evaluated environmental factors involved in the etiology of PBC. In this mini-review, we discuss the environmental basis of autoimmunity in PBC as a representative autoimmune disease.

What is PBC?

PBC is a chronic cholestatic liver disease that predominantly affects middle-aged females, but can occur in adults of all ages.[11] Histologically, PBC manifests as chronic non-suppurative destructive cholangitis (Figure 1) with formation of granulomas in the liver, degeneration and necrosis of biliary epithelial cells (BECs), and disappearance of small or medium-sized intrahepatic bile ducts, which lead to chronic and progressive cholestasis. Although the etiology of PBC has not been fully defined, it is evident that autoimmune reactivity against intrahepatic BECs plays a key role in disease pathology.[9,12] Since subjective symptoms are uncommon in patients with PBC, the disease is often detected incidentally during random blood testing. However, a substantial number of patients experience a variety of symptoms, including pruritus, fatigue, dryness, and body pain. Other autoimmune diseases such as rheumatoid arthritis, Sjogren's syndrome, and chronic thyroiditis frequently coexist in patients with PBC. As the disease progresses, jaundice and other decompensating events of the liver develop, ultimately resulting in liver failure and the need for liver transplantation. Ursodeoxycholic acid (UDCA), which was previously the only approved drug for PBC, effectively extended liver transplantation-free survival in several clinical trials in the 1990s. Thereafter, the use of UDCA as a first-line drug, with the introduction of AMAs testing in clinical settings, enabled PBC patients to be diagnosed in asymptomatic stages before progression to cirrhosis.[13] Thus, the name of the disease was changed from primary biliary "cirrhosis" to primary biliary "cholangitis."[14]

Genetic background – "Bad gene"

PBC results from a combination of "bad genes and bad luck"; individuals with a genetic predisposition to the disease develop PBC due to environmental triggers. The relevance of genetic predisposition in PBC is evident with familial clustering, in which the prevalence of PBC patients increases among first-degree relatives and siblings of index patient.[15–18] The concordance rate of PBC is 63% in monozygotic twins, which is higher than that of other autoimmune diseases.[19] Case-control studies and studies using modern technology such as GWAS have shown that HLA alleles possess the strongest link to PBC susceptibility, with more than 40 non-HLA alleles contributing as well.[20] Although these risk alleles differ among studies and populations, pathways involving identified genes are largely shared among populations and related to antigen presentation and production of interleukin (IL)-12 (*IRF5*, *SOCS1*, *TNFAIP3*, *NFκB*, *IL-12A*), activation of T cells and interferon (IFN)-γ production (*TNFSF15*, *IL12R*, *TYK2*, *STAT4*, *SOCS1*, *NFκB*, *TNFAIP3*), and activation of B cells and production of immunoglobulins (*POU2AF1*, *SPIB*, *PRKCB*, *IKZF3*, *ARID3A*). Thus, these immune pathways are thought to be important in the pathogenesis of PBC. However, as with other autoimmune diseases, GWAS have not been able to identify a "smoking gun" of the genetic basis for PBC, suggesting that environmental factors, particularly epigenetics, play a crucial role.[21]

Environmental basis – "Bad luck"

It is clear that PBC risk is not defined exclusively by genetic predisposition. Only two-thirds of monozygotic twins share PBC and not all family members develop PBC, even in a family tree with heavy clustering.[15,19] According to recent epidemiological studies, the risk of developing PBC in first-degree relatives of the indicated patient was relatively low during eight years of follow-up.[22] Therefore, environmental factors likely play a significant role in the development of PBC in addition to genetic factors. Researchers, including our group, have mainly focused on the implications of bacterial infection and xenobiotics

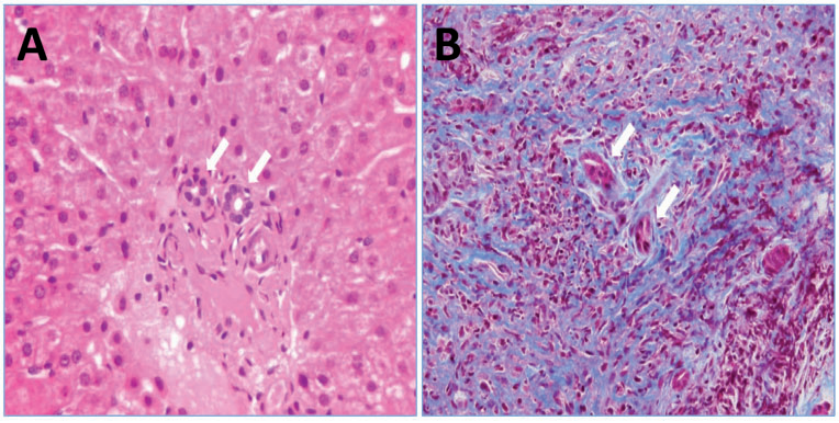

Figure 1. Liver histology from healthy individuals (a) and PBC patients (b). Note intact biliary epithelial cells (arrows) in livers from healthy individuals (a) but the bile ducts are collapsed, BECs are irregularly shaped (arrows), with massive infiltrates of lymphocytes aggregated in the vicinity of BECs in PBC liver (hematoxylin and eosin staining). (A color version of this figure is available in the online journal.)

as candidate environmental factors. These may provide insight into the precise mechanism of tolerance breakdown against autoantigens leading to the production of AMAs.

AMAs

AMAs are the most disease-specific autoantibodies in human immunopathology and are detected in 90–95% of PBC patients.[23,24] The immunodominant epitopes of AMAs were identified as the pyruvate dehydrogenase complex E2 subunit (PDC-E2) by cDNA cloning.[25] AMAs recognize a family of enzymes in the 2-oxo-acid dehydrogenase complex (2-OADC) located in the inner mitochondrial membrane, including PDC-E2, branched-chain 2-OADC, 2-oxo-glutaric acid dehydrogenase complex, and dihydrolipoamide dehydrogenase binding protein.[26] These E2 enzymes all share a common protein structure consisting of an N-terminal domain with a single or multiple attachment sites to a lysine (^{173}K in mammalian PDC-E2) in lipoic acid (LA) (Table 1). The dominant AMA epitope was mapped within the inner lipoyl domains of the enzyme complex[27–29] and the amino acid residues critical for maintaining the structural integrity of the PDC-E2 lipoyl domain have been determined by site-directed mutagenesis.[30] Interestingly, epitopes of both autoreactive PDC-E2-specific $CD4^+$ and $CD8^+$ T cells, which are crucial to the pathogenesis of PBC, were also mapped in the same region of human PDC-E2.[31,32] This suggests that AMAs recognizing mitochondrial autoantigens, particularly PDC-E2, are not simply serological markers for diagnosis but important drivers of PBC immunopathology as well.[33]

Bacterial infection

Large-scale case-control studies have consistently found an association between PBC and urinary tract infections caused by *Escherichia coli*.[16,34–36] Human PDC-E2 is molecularly similar to *E. coli* PDC-E2 (Table 1). In particular, the entire ExDK sequence, reported to be essential for recognition of $CD4^+$ PDC-E2-specific T cells, is shared by both human and *E. coli* PDC-E2.[32] Thus, an *E. coli* infection may trigger the breakdown of immunological tolerance against human PDC-E2 through molecular mimicry and cross-reactivity.

Another bacterium that may be involved in the etiology of PBC through cross-reactivity is *Novosphingobium aromaticivorans*, a ubiquitous xenobiotic-metabolizing bacterium that has been identified by protein homology searching of human PDC-E2 (Table 1). Immunological studies demonstrated that *N. aromaticivorans* contains lipoylated proteins, which are 100 to 1000-fold more reactive with PBC patient sera than those of *E. coli*.[37] *N. aromaticivorans* is thus a potential trigger for PBC due to its higher reactivity compared to *E. coli*, capacity to metabolize xenobiotics, and presence in feces.

Besides bacteria, the involvement of endogenous retroviruses in PBC immunopathology has also been studied. Nucleic acid sequences of the human betaretrovirus homologous to those of the mouse mammary tumor virus and human breast cancer-derived retrovirus have been cloned from the lymph nodes of PBC patients.[38]

Xenobiotics

Extensive amount of epidemiological data indicate that xenobiotics are likely involved in the development of PBC. Case-control studies have analyzed various lifestyle factors involved in PBC, and found that frequent use of nail polish was associated with increased PBC susceptibility.[35] Furthermore, PBC patients tend to be geographically concentrated near toxic waste sites.[39–41] Since AMAs are known to be important in the immunopathology of PBC, researchers also examined environmental mimotopes in the form of xenobiotics. A detailed, quantitative structure–activity relationship analysis of 107 potential xenobiotic mimics coupled to the lysine residue of the immunodominant 15-amino-acid peptide of the PDC-E2 inner lipoyl domain (ILD) revealed that 2-octynamide, the conjugate derived from 2-octynoic acid (2-OA) present in cosmetics, lipsticks, and some chewing gums, stood out in both its quantitative structure–activity relationship analysis and reactivity with PBC sera.[42] Furthermore, another xenobiotic, 2-nonyamide has the optimal chemical structure for xenobiotic modification of the epitope, which has been demonstrated by enhanced recognition with AMA-positive PBC sera.[43] Significant molecular mimicry between lipoamide and 2-nonynamide was indeed observed (Figure 2). These findings suggest that PDC-E2 modified with chemicals abundantly found in daily life can generate immunogenic neoantigens that breach tolerance in PBC. Furthermore, monoclonal antibodies cross-reactive with both native PDC-E2 and 2-OA also recognize LA.[44] Recent structural studies have demonstrated that the conformation of PDC-E2 ILD is altered when conjugated with 2OA and not LA,[30,45] which further supports the

Table 1. Molecular mimicry and immunodominant epitopes of human PDC-E2 amino acid residue 155–185.[a]

Human PDC-E2	K	V	G	E	K	L	S	E	G	D	L	L	A	E	I	E	T	D	K	A	T	I	G	F	E	V	Q	E	E	G	Y
B cell epitope	K	V	G	E	K	L	S	E	G	D	L	L	A	E	I	E	T	D	K	A	T	I	G	F	E	V	Q	E	E	G	Y
CD4 T cell epitope	K	V	G	E	K	L	S	E	*G*	*D*	*L*	*L*	*A*	*E*	*I*	*E*	*T*	*D*	*K*	*A*	*T*	*I*	G	F	E	V	Q	E	E	G	Y
CD8 T cell epitope	K	V	G	E	*K*	*L*	*S*	*E*	*G*	*D*	*L*	*L*	*A*	E	I	E	T	D	K	A	T	I	G	F	E	V	Q	E	E	G	Y
E coli L1	-	-	-	D	-	V	E	A	E	Q	S	-	I	T	V	-	G	-	-	-	S	M	E	V	P	S	P	Q	A	-	I
E coli L2	-	-	-	D	-	V	E	A	E	Q	S	-	I	T	V	-	G	-	-	-	S	M	E	V	P	A	P	F	A	-	T
E. coli L3	-	-	-	D	-	V	A	A	E	Q	S	-	I	T	V	-	G	-	-	-	S	M	E	V	P	A	P	F	A	-	V
Novosphongobium aromaticivorans	-	A	-	D	E	V	R	S	-	-	I	-	A	E	I	-	T	-	-	-	T	M	E	F	E	A	V	D	E	-	V

[a]The lysine residue K denotes ^{173}lysine; the lipoic acid binding site of human PDC-E2.

Note: The CD4 T cell and CD8 T cell epitope are BOLDED in italics.

"-" denotes the *E. coli* PDC-E2 and *N. aromarticivorans* PDC-E2 amino acid residues that are identical with human PDC-E2 lipoyl domain.

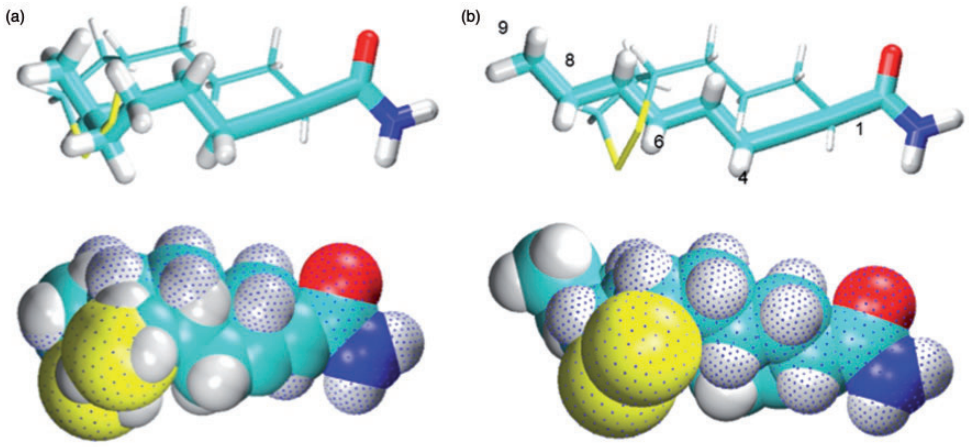

Figure 2. Molecular mimicry between lipoamide and 2-nonynamide. Superimposed models of lipoamide (dotted) and 2-nonynamide, in space-filled and bond representations with 2-nonynamide in either "corkscrew" (a) or straight chain conformation (b). (A color version of this figure is available in the online journal.)

Table 2. Characteristics of PBC mouse models.

	Spontaneous models				**Induced model**
	NOD.c3c4	**dnTGFbRII**	**IL-2Rα $^{-/-}$**	**ARE Del$^{-/-}$**	**2-OA–BSA immunized**
Female dominance	Yes	No	No	Yes	No
Cholestasis	–	+	–	+	+
AMA seropositivity	50–60%	100%	100%	100%	100%
Portal inflammation	+++	+++	+++	Yes	+
Granulomas	+	–	–	+	+
Other features	Biliary polycystic lesions	Moderate colitis	Severe anemia, inflammatory bowel diseases, and short life span		Peritonitis
References	(58)	(59)	(60)	(61,62)	(55)

PBC: primary biliary cholangitis; 2-OA: 2-octynoic acid; BSA: bovine serum albumin; ARE: adenylate uridine-rich element; AMA: anti-mitochondrial autoantibody.

hypothesis that xenobiotically modified LA is the initial target of autoimmunity in PBC.

Other candidate environmental factors

Genome-wide epigenetic analysis revealed significant differences in methylation profiles, copy number variation, and gene expression in three monozygotic twins and eight sibling pairs discordant for PBC.[46] Moreover, aberrant demethylation of the CXCR3 promoter of the X chromosome was noted in patients with PBC.[47] However, since these findings are only descriptive, further studies are needed to determine the etiological implications of epigenetics.[48] Finally, involvement of gut microbiota in the pathogenesis of autoimmune disease has been suggested as well.[49,50] One comparative study found gut dysbiosis in PBC patients, which was partially resolved with UDCA treatment.[51]

Xenobiotic-triggered murine models of PBC

In addition to *in vitro* studies, murine models are important for understanding the etiology and natural history of PBC. Since patients with newly diagnosed PBC are well beyond the initial stage of loss of tolerance, there is likely a long latency period between the appearance of autoantibodies and clinical symptoms/disease.[52] Therefore, animal models that reflect many important aspects of the disease are needed to explore the initiating events and interactions between genetic and environmental factors. The animal model should have the same physiological mechanisms observed in human PBC, such as female predominance, chronic cholestasis, AMA production, bile duct involvement, and histological features including lymphocyte infiltration into the liver.[53] To date, several murine models that develop autoimmune cholangitis resembling PBC have been established spontaneously or through xenobiotic induction (Table 2). These mice share some of the important clinical phenotypes of PBC.[54] In particular, immunization of mice with 2-OA, a potential environmental trigger for PBC, induced autoimmune cholangitis mimicking PBC and AMA seropositivity.

Our group immunized C57BL/6 mice with 2-OA conjugated to bovine serum albumin (BSA) and found that anti-PDC-E2 antibodies were developed as early as four weeks after 2OA-BSA immunization, indicating a loss of tolerance to PDC-E2 with xenobiotic immunization. In addition, these mice demonstrated portal infiltration of $CD4^+$ and $CD8^+$ T cells, granulomas, and elevated tumor necrosis factor-α and IFN-γ expression levels.[55] Using several unique gene-deleted mice immunized with 2-OA-BSA,[56] we revealed that both IL-12/T helper type 1 (Th1) and IL-23/Th17 were involved in autoimmune cholangitis.

The IL-12/Th1 signaling pathway elicited the pathology, whereas deletion of IFN- γ prevented autoimmune cholangitis. Although these mice lack several characteristics of PBC such as female dominance, they clearly demonstrate the etiological importance of 2-OA in PBC.

Concluding remarks

The prevalence of autoimmune diseases including PBC is increasing worldwide,[57] possibly due to increased amount of environmental exposure to xenobiotics. Large-scale, multi-center case-control studies are needed to identify xenobiotic factors and examine their roles in the development of autoimmune diseases. International collaboration in this subject should also take into account the ethnic diversity in genetic predisposition. Immunological investigations and establishment of relevant animal models will be critical to decipher how environmental factors play a role in natural history of autoimmune diseases.

Authors' contributions: AT and PSCL drafted the paper and MEG critically reviewed the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

1. Doherty DG. Immunity, tolerance and autoimmunity in the liver: a comprehensive review. *J Autoimmun* 2016;**66**:60–75
2. Lleo A, Invernizzi P, Gao B, Podda M, Gershwin ME. Definition of human autoimmunity–autoantibodies versus autoimmune disease. *Autoimmun Rev* 2010;**9**:A259–66
3. Gianchecchi E, Fierabracci A. Gene/environment interactions in the pathogenesis of autoimmunity: new insights on the role of toll-like receptors. *Autoimmun Rev* 2015;**14**:971–83
4. Pollard KM. Environment, autoantibodies, and autoimmunity. *Front Immunol* 2015;**6**:60
5. Webb GJ, Hirschfield GM. Using GWAS to identify genetic predisposition in hepatic autoimmunity. *J Autoimmun* 2016;**66**:25–39
6. He XS, Gershwin ME, Ansari AA. Checkpoint-based immunotherapy for autoimmune diseases – opportunities and challenges. *J Autoimmun* 2017;**79**:1–3
7. Miller FW, Alfredsson L, Costenbader KH, Kamen DL, Nelson LM, Norris JM, De Roos AJ. Epidemiology of environmental exposures and human autoimmune diseases: findings from a National Institute of Environmental Health Sciences Expert Panel Workshop. *J Autoimmun* 2012;**39**:259–71
8. Ji J, Sundquist J, Sundquist K. Gender-specific incidence of autoimmune diseases from national registers. *J Autoimmun* 2016;**69**:102–6
9. Gershwin ME, Ansari AA, Mackay IR, Nakanuma Y, Nishio A, Rowley MJ, Coppel RL. Primary biliary cirrhosis: an orchestrated immune response against epithelial cells. *Immunol Rev* 2000;**174**:210–25
10. Gershwin ME, Mackay IR. The causes of primary biliary cirrhosis: convenient and inconvenient truths. *Hepatology* 2008;**47**:737–45
11. Carey EJ, Ali AH, Lindor KD. Primary biliary cirrhosis. *Lancet* 2015;**386**:1565–75
12. Hisamoto S, Shimoda S, Harada K, Iwasaka S, Onohara S, Chong Y, Nakamura M, Bekki Y, Yoshizumi T, Ikegami T, Maehara Y, He XS, Gershwin ME, Akashi K. Hydrophobic bile acids suppress expression of AE2 in biliary epithelial cells and induce bile duct inflammation in primary biliary cholangitis. *J Autoimmun* 2016;**75**:150–60
13. Mousa HS, Carbone M, Malinverno F, Ronca V, Gershwin ME, Invernizzi P. Novel therapeutics for primary biliary cholangitis: toward a disease-stage-based approach. *Autoimmun Rev* 2016;**15**:870–6
14. Beuers U, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, Ma X, Mackay IR, Pares A, Tanaka A, Vierling JM, Poupon R. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. *Gastroenterology* 2015;**149**:1627–9
15. Abu-Mouch S, Selmi C, Benson GD, Kenny TP, Invernizzi P, Zuin M, Podda M, Rossaro L, Gershwin ME. Geographic clusters of primary biliary cirrhosis. *Clin Dev Immunol* 2003;**10**:127–31
16. Corpechot C, Chretien Y, Chazouilleres O, Poupon R. Demographic, lifestyle, medical and familial factors associated with primary biliary cirrhosis. *J Hepatol* 2010;**53**:162–9
17. Mantaka A, Koulentaki M, Chlouverakis G, Enele-Melono JM, Darivianaki A, Tzardi M, Kouroumalis EA. Primary biliary cirrhosis in a genetically homogeneous population: disease associations and familial occurrence rates. *BMC Gastroenterol* 2012;**12**:110
18. Yanagisawa M, Takagi H, Takahashi H, Uehara M, Otsuka T, Yuasa K, Hosonuma K, Mori M. Familial clustering and genetic background of primary biliary cirrhosis in Japan. *Dig Dis Sci* 2010;**55**:2651–8
19. Selmi C, Mayo M, Bach N, Ishibashi H, Invernizzi P, Gish R, Gordon S, Wright H, Zweiban B, Podda M, Gershwin M. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. *Gastroenterology* 2004;**127**:485–92
20. Gulamhusein AF, Juran BD, Lazaridis KN. Genome-wide association studies in primary biliary cirrhosis. *Semin Liver Dis* 2015;**35**:392–401
21. Xiang Z, Yang Y, Chang C, Lu Q. The epigenetic mechanism for discordance of autoimmunity in monozygotic twins. *J Autoimmun* 2017;**83**:43–50
22. Gulamhusein AF, Juran BD, Atkinson EJ, McCauley B, Schlicht E, Lazaridis KN. Low incidence of primary biliary cirrhosis (PBC) in the first-degree relatives of PBC probands after 8 years of follow-up. *Liver Int* 2016;**36**:1378–82
23. Van de Water J, Gershwin M, Leung P. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. *J Exp Med* 1988;**167**:1791–9
24. Oertelt S, Rieger R, Selmi C, Invernizzi P, Ansari AA, Coppel RL, Podda M, Leung PS, Gershwin MEA. sensitive bead assay for antimitochondrial antibodies: chipping away at AMA-negative primary biliary cirrhosis. *Hepatology* 2007;**45**:659–65
25. Gershwin M, Mackay I, Sturgess A, Coppel R. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. *J Immunol* 1987;**138**:3525–31
26. Dubel L, Tanaka A, Leung P, Van de Water J, Coppel R, Roche T, Johanet C, Motokawa Y, Ansari A, Gershwin M. Autoepitope mapping and reactivity of autoantibodies to the dihydrolipoamide dehydrogenase-binding protein (E3BP) and the glycine cleavage proteins in primary biliary cirrhosis. *Hepatology* 1999;**29**:1013–8
27. Leung P, Chuang D, Wynn R, Cha S, Danner D, Ansari A, Coppel R, Gershwin M. Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. *Hepatology* 1995;**22**:505–13
28. Moteki S, Leung P, Dickson E, Van Thiel D, Galperin C, Buch T, Alarcon-Segovia D, Kershenobich D, Kawano K, Coppel R, Gershwin M. Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. *Hepatology* 1996;**23**:436–44
29. Surh C, Coppel R, Gershwin M. Structural requirement for autoreactivity on human pyruvate dehydrogenase-E2, the major autoantigen of primary biliary cirrhosis. Implication for a conformational autoepitope. *J Immunol* 1990;**144**:1321–8

30. Wang J, Budamagunta MS, Voss JC, Kurth MJ, Lam KS, Lu L, Kenny TP, Bowlus C, Kikuchi K, Coppel RL, Ansari AA, Gershwin ME, Leung PS. Antimitochondrial antibody recognition and structural integrity of the inner lipoyl domain of the E2 subunit of pyruvate dehydrogenase complex. *J Immunol* 2013;**191**:2126–33
31. Kita H, Lian ZX, Van de Water J, He XS, Matsumura S, Kaplan M, Luketic V, Coppel RL, Ansari AA, Gershwin ME. Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. *J Exp Med* 2002;**195**:113–23
32. Shimoda S, Nakamura M, Ishibashi H. HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune disease. *J Exp Med* 1995;**181**:1835–45
33. Chung BK, Guevel BT, Reynolds GM, Gupta Udatha DB, Henriksen EK, Stamataki Z, Hirschfield GM, Karlsen TH, Liaskou E. Phenotyping and auto-antibody production by liver-infiltrating B cells in primary sclerosing cholangitis and primary biliary cholangitis. *J Autoimmun* 2017;**77**:45–54
34. Burroughs A, Rosenstein I, Epstein O, Hamilton-Miller J, Brumfitt W, Sherlock S. Bacteuria and primary biliary cirrhosis. *Gut* 1984;**25**:133–7
35. Gershwin ME, Selmi C, Worman HJ, Gold EB, Watnik M, Utts J, Lindor KD, Kaplan MM, Vierling JM. Group UPE. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. *Hepatology* 2005;**42**:1194–202
36. Howel D, Fischbacher CM, Bhopal RS, Gray J, Metcalf JV, James OF. An exploratory population-based case-control study of primary biliary cirrhosis. *Hepatology* 2000;**31**:1055–60
37. Selmi C, Balkwill D, Invernizzi P, Ansari A, Coppel R, Podda M, Leung P, Kenny T, Van D, Water J, Nantz M, Kurth M, Gershwin M. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. *Hepatology* 2003;**38**:1250–7
38. Vergani D, Bogdanos DP, Baum H. Unusual suspects in primary biliary cirrhosis. *Hepatology* 2004;**39**:38–41
39. Ala A, Stanca CM, Bu-Ghanim M, Ahmado I, Branch AD, Schiano TD, Odin JA, Bach N. Increased prevalence of primary biliary cirrhosis near Superfund toxic waste sites. *Hepatology* 2006;**43**:525–31
40. McNally RJ, James PW, Ducker S, Norman PD, James OF. No rise in incidence but geographical heterogeneity in the occurrence of primary biliary cirrhosis in north East England. *Am J Epidemiol* 2014;**179**:492–8
41. Prince MI, Chetwynd A, Diggle P, Jarner M, Metcalf JV, James OF. The geographical distribution of primary biliary cirrhosis in a well-defined cohort. *Hepatology* 2001;**34**:1083–8
42. Amano K, Leung P, Rieger R, Quan C, Wang X, Marik J, Suen Y, Kurth M, Nantz M, Ansari A, Lam K, Zeniya M, Matsuura E, Coppel R, Gershwin M. Chemical xenobiotics and mitochondrial autoantigens in primary biliary cirrhosis: identification of antibodies against a common environmental, cosmetic, and food additive, 2-octynoic acid. *J Immunol* 2005;**174**:5874–83
43. Rieger R, Leung PS, Jeddeloh MR, Kurth MJ, Nantz MH, Lam KS, Barsky D, Ansari AA, Coppel RL, Mackay IR, Gershwin ME. Identification of 2-nonynoic acid, a cosmetic component, as a potential trigger of primary biliary cirrhosis. *J Autoimmun* 2006;**27**:7–16
44. Tanaka T, Zhang W, Sun Y, Shuai Z, Chida A, Kenny TP, Yang GX, Sanz I, Ansai A, Bowlus CL, Ippolito GC, Coppel RL, Okazaki K, He XS, Leung PSC, Gershwin ME. Autoreactive monoclonal antibodies from patients with primary biliary cholangitis recognize environmental xenobiotics. *Hepatology* 2017;**66**:885–95
45. Shuai Z, Wang J, Badamagunta M, Choi J, Yang G, Zhang W, Kenny TP, Guggenheim K, Kurth MJ, Ansari AA, Voss J, Coppel RL, Invernizzi P, Leung PSC, Gershwin ME. The fingerprint of antimitochondrial antibodies and the etiology of primary biliary cholangitis. *Hepatology* 2017;**65**:1670–82
46. Selmi C, Cavaciocchi F, Lleo A, Cheroni C, D, Francesco R, Lombardi SA, D, Santis M, Meda F, Raimondo MG, Crotti C, Folci M, Zammataro L, Mayo MJ, Bach N, Shimoda S, Gordon SC, Miozzo M, Invernizzi P, Podda M, Scavelli R, Martin MR, Seldin MF, Lasalle JM, Gershwin ME. Genome-wide analysis of DNA methylation, copy number variation, and gene expression in monozygotic twins discordant for primary biliary cirrhosis. *Front Immunol* 2014;**5**:128
47. Lleo A, Zhang W, Zhao M, Tan Y, Bernuzzi F, Zhu B, Liu Q, Tan Q, Malinverno F, Valenti L, Jiang T, Tan L, Liao W, Coppel R, Invernizzi P, Lu Q, Adams DH, Gershwin ME. DNA methylation profiling of the X chromosome reveals an aberrant demethylation on CXCR3 promoter in primary biliary cirrhosis. *Clin Epigenet* 2015;**7**:61
48. Xie YQ, Ma HD, Lian ZX. Epigenetics and primary biliary cirrhosis: a comprehensive review and implications for autoimmunity. *Clin Rev Allergy Immunol* 2016;**50**:390–403
49. Chen B, Sun L, Zhang X. Integration of microbiome and epigenome to decipher the pathogenesis of autoimmune diseases. *J Autoimmun* 2017;**83**:31–42
50. Li Y, Tang R, Leung PSC, Gershwin ME, Ma X. Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases. *Autoimmun Rev* 2017;**16**:885–96
51. Tang R, Wei Y, Li Y, Chen W, Chen H, Wang Q, Yang F, Miao Q, Xiao X, Zhang H, Lian M, Jiang X, Zhang J, Cao Q, Fan Z, Wu M, Qiu D, Fang JY, Ansari A, Gershwin ME, Ma X. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut* 2017. Epub ahead of print 17 February. doi: 10.1136/gutjnl-2016-313332.
52. Ma WT, Chang C, Gershwin ME, Lian ZX. Development of autoantibodies precedes clinical manifestations of autoimmune diseases: a comprehensive review. *J Autoimmun* 2017;**83**:95–112
53. Wang J, Yang GX, Tsuneyama K, Gershwin ME, Ridgway WM, Leung PS. Animal models of primary biliary cirrhosis. *Semin Liver Dis* 2014;**34**:285–96
54. Katsumi T, Tomita K, Leung PS, Yang GX, Gershwin ME, Ueno Y. Animal models of primary biliary cirrhosis. *Clin Rev Allergy Immunol* 2015;**48**:142–53
55. Wakabayashi K, Lian ZX, Leung PS, Moritoki Y, Tsuneyama K, Kurth MJ, Lam KS, Yoshida K, Yang GX, Hibi T, Ansari AA, Ridgway WM, Coppel RL, Mackay IR, Gershwin ME. Loss of tolerance in C57BL/6 mice to the autoantigen E2 subunit of pyruvate dehydrogenase by a xenobiotic with ensuing biliary ductular disease. *Hepatology* 2008;**48**:531–40
56. Kawata K, Tsuda M, Yang GX, Zhang W, Tanaka H, Tsuneyama K, Leung P, He XS, Knechtle S, Ansari AA, Coppel RL, Gershwin ME. Identification of potential cytokine pathways for therapeutic intervention in murine primary biliary cirrhosis. *PLoS One* 2013;**8**:e74225
57. Boonstra K, Beuers U, Ponsioen CY. Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review. *J Hepatol* 2012;**56**:1181–8
58. Irie J, Wu Y, Wicker LS, Rainbow D, Nalesnik MA, Hirsch R, Peterson LB, Leung PS, Cheng C, Mackay IR, Gershwin ME, Ridgway WM. NOD.c3c4 congenic mice develop autoimmune biliary disease that serologically and pathogenetically models human primary biliary cirrhosis. *J Exp Med* 2006;**203**:1209–19
59. Oertelt S, Lian ZX, Cheng CM, Chuang YH, Padgett KA, He XS, Ridgway WM, Ansari AA, Coppel RL, Li MO, Flavell RA, Kronenberg M, Mackay IR, Gershwin ME. Anti-mitochondrial antibodies and primary biliary cirrhosis in TGF-beta receptor II dominant-negative mice. *J Immunol* 2006;**177**:1655–60
60. Wakabayashi K, Lian ZX, Moritoki Y, Lan RY, Tsuneyama K, Chuang YH, Yang GX, Ridgway W, Ueno Y, Ansari AA, Coppel RL, Mackay IR, Gershwin ME. IL-2 receptor alpha(-/-) mice and the development of primary biliary cirrhosis. *Hepatology* 2006;**44**:1240–9
61. Bae HR, Hodge DL, Yang GX, Leung PSC, Chodisetti SB, Valencia JC, Sanford M, Fenimore JM, Rahman ZSM, Tsuneyama K, Norman GL, Gershwin ME, Young HA. The interplay of type I and type II interferons in murine autoimmune cholangitis as a basis for sex-biased autoimmunity. *Hepatology* 2017. Epub ahead of print 16 September. doi: 10.1002/hep.29524.
62. Bae HR, Leung PS, Tsuneyama K, Valencia JC, Hodge DL, Kim S, Back T, Karwan M, Merchant AS, Baba N, Feng D, Park O, Gao B, Yang GX, Gershwin ME, Young HA. Chronic expression of interferon-gamma leads to murine autoimmune cholangitis with a female predominance. *Hepatology* 2016;**64**:1189–201