MRGPRX4 in Cholestatic Pruritus

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Abstract

Pruritus (itch) is a debilitating symptom in liver diseases with cholestasis, which severely affects patients' quality of life. Limited treatment options are available for cholestatic itch, largely due to the incomplete understanding of the underlying molecular mechanisms. Several factors have been proposed as pruritogens for cholestatic itch, such as bile acids, bilirubin, lysophosphatidic acid, and endogenous opioids. Recently, two research groups independently identified Mas-related G protein-coupled receptor X4 (MRGPRX4) as a receptor for bile acids and bilirubin and demonstrated its likely role in cholestatic itch. This discovery not only opens new avenues for understanding the molecular mechanisms in cholestatic itch but provides a promising target for developing novel anti-itch treatments. In this review, we summarize the current theories and knowledge of cholestatic itch, emphasizing MRGPRX4 as a bile acid and bilirubin receptor mediating cholestatic itch in humans. We also discuss some future perspectives in cholestatic itch research.

Keywords

- MRGRPX4
- cholestasis
- pruritus

Pruritus (itch) commonly occurs in patients with cholestatic liver diseases. Although cholestatic itch has been described for more than 2,000 years,[1] the underlying mechanisms have not been well understood. Current treatments for cholestatic itch include ursodeoxycholic acid, cholestyramine, and rifampicin[2]; however, these compounds are usually effective for some but not all patients and have considerable side effects. In addition, a small handful of compounds are currently undergoing clinical trials for the treatment of cholestatic itch, such as agonists of peroxisome proliferator-activated receptors,[3,4] and inhibitors of ileal bile acid transporters.[5,6] To develop more effective treatments for cholestatic itch, understanding molecular mechanisms underlying cholestatic itch is a critical step. In this review, we summarize the current theories and knowledge about cholestatic itch by focusing on the newly identified bile acid receptor Mas-related G protein-coupled receptor X4 (MRGPRX4).

Cholestasis and Itch in Liver Diseases

Cholestasis is associated with a variety of pathological liver conditions in which the flow of bile is reduced or completely blocked.[7] Metabolites in the bile, including bile acids and bilirubin, build up in the liver, spill into the circulation, and ultimately accumulate in other tissues and organs, such as the cornea and the skin.[8] Bilirubin is pigmented and high levels result in jaundice, the yellow-tinged color changes the skin and eyes.[9] There are two types of cholestatic conditions, intrahepatic and extrahepatic cholestasis. Intrahepatic cholestasis is characterized by the evidence of retention of biliary constituents without demonstrable anatomical obstruction of the biliary tree, in which bile formation is disrupted by defects within the parenchymal cells of the liver, the hepatocytes, due to genetic factors, autoimmune hepatitis, pregnancy, or medications.[7,8] Extrahepatic

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Table 1 Cholestatic itch in liver diseases and their prevalence

Liver disease	Prevalence (%)	Sample size	Reference
Primary biliary cirrhosis (PBC)	51.4	72	Oeda et al, 2018[67]
	69.3	49	Koulentaki et al, 2006[68]
	69.3	238	Rishe et al, 2008[69]
	65.5[2]	180	Tanaka et al, 2016[70]
Chronic viral hepatitis C (HCV)	45.9	366	Oeda et al, 2018[67]
	31.0	171	Maticic et al, 2008[71]
	15	100	Cribier et al, 1998[72]
	15	1,614	Cacoub et al, 1999[73]
	38.9	262	Oeda et al, 2018[67]
Nonalcoholic fatty liver disease (NAFLD)	44.7	338	Oeda et al, 2018[67]
Active viral hepatitis B infection	40.6	175	Oeda et al, 2018[67]
Alcoholic liver disease (ALD)	34.2	76	Oeda et al, 2018[67]
Autoimmune hepatitis (AIH)	24.3	70	Oeda et al, 2018[67]
Intrahepatic cholestasis of pregnancy (ICP)	79.2	340	Lee et al, 2006[11]
Inactive viral hepatitis B carrier	22.2	54	Oeda et al, 2018[67]

cholestasis results from a clear mechanical obstruction in the bile duct system, such as by gallstones or malignancy.[7,8]

Itch is a prevalent symptom in liver diseases with cholestasis, such as primary biliary cholangitis (PBC), primary sclerosing cholangitis, obstructive choledocholithiasis, viral hepatitis, intrahepatic cholestasis of pregnancy (ICP).[10–12] The exact prevalence of itch in liver diseases varies in different pathological conditions and in different studies. In PBC, the incidence of itching can be as high as 70%, and severe itch is an indication for liver transplantation. ►**Table 1** summarizes the cholestatic itch prevalence from a few different studies.

To date, a few molecules have been proposed as the pruritogens of cholestatic itch, including bile acids, bilirubin, lysophosphatidic acid (LPA), BAM8–22, substance P, and endogenous opioids.[13,14] With respect to the cognate receptors for these potential pruritogens, some of them have been proposed, albeit primarily based on studies using rodent models. For example, the membrane-bound bile acid receptor TGR5 has been reported to mediate bile acid-induced itch in mice[15,16]; LPA receptors are reported to be present in mouse dorsal root ganglion (DRG) neurons and are reported to activate a signaling pathway in DRG neurons[17–19]; BAM8–22 and substance P are implicated to involve in cholestatic itch through Mas-related G protein-coupled receptors (MRGPRs)[20,21]; opioid receptors, widely expressed in central and peripheral nervous systems, are implicated to mediate itch in cholestatic conditions due to the increased endogenous opioid levels.[22,23] However, whether or not these receptors mediate cholestatic itch in human requires further studies.

Interruption of enterohepatic recirculation reduces pruritus, indicating that some molecules within bile play a critical role in cholestatic itch.[24–27] Based on their elevation in cholestatic conditions, bile acids and bilirubin have been proposed to be pruritogens for cholestatic itch for many years.[28,29] However, the direct evidence that they are itch-inducing substances has been lacking and the molecular receptor(s) through which they induce itch also has remained unclear. Recently, Xinzhong Dong's laboratory[30,31] and a collaborative group of Yulong Li's at Peking University and Wenqin Luo's laboratory at the University of Pennsylvania[32] independently identified MRGPRX4 as bile acids and bilirubin receptor in human and homolog MRGPRA1 as bilirubin receptor in mice for cholestatic itch.

MRGPR Family and Itch Sensation

MRGPR is a GPCR subfamily. In a human, there are eight MRGPR members, X1-X4, D, E, F, G. In mice, the MRGPR member D, E, F, and G are concordant with human ones.[33] However, the mouse A, B, and C families, which are closely mapped to human X family, have an atypical expansion. Mice have approximately 50 members in A, B, and C families, half of which are pseudogenes.[34] MRGPRs display specific expression in a subset of small-diameter primary somatosensory neurons (dorsal root and trigeminal ganglion neurons) that mediate pain, itch, and thermal sensation.[34,35] Xinzhong Dong and his colleagues pioneered the identification and functional determination of the MRGPR family. They demonstrated that several mouse and human MRGPR members—Mm.MRGPRA3, Mm.MRGPRB2, Mm.MRGPRC11, Mm.MRGPRD, Hs.MRGPRX1, and Hs.MRGPRD—are itch receptors.[36–38] Other groups also help to identify novel ligand MRGPR pairs and elucidate their functions in itch sensation (Summarized in ►**Fig. 1** and ►**Table 2**).

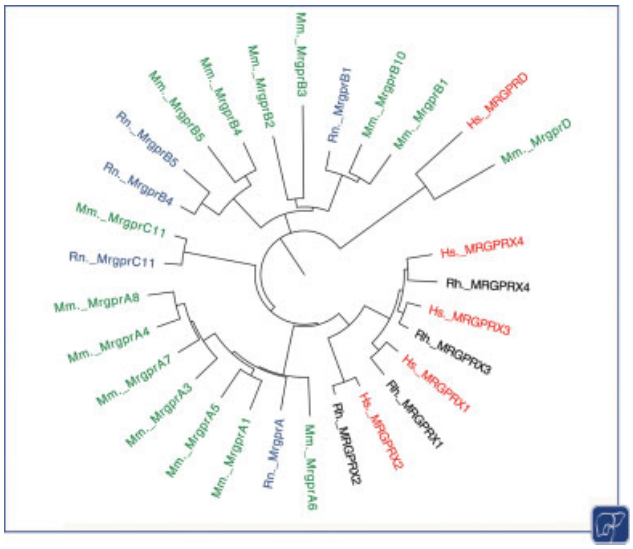

Fig. 1 MRGPR evolutionary tree. Phylogenetic analysis of mouse (Mm, *green*), rat (Rn, *blue*), rhesus monkey (Rh, *black*), and human (Hs, *red*) Mas-related GPCR (Mrg) family members. Phylogenetic tree generated from UniProt sequences aligned by the European Bioinformatics Institute (EMBL-EBI) Clustal Omega tool[81] and constructed by the software FigTree version 1.4.4.[82] The scale indicates the number of substitutions per amino acid in the sequence. MRGPR, Mas-related G protein-coupled receptor.

Identification of MRGPRX4 as a Receptor for Bile Acids and Bilirubin

To identify the molecular receptor mediating cholestatic itch, Meixiong et al first focused on bilirubin as the potential pruritogen, which could trigger itch sensation in mice by subcutaneous injection.[30] They screened 12 mouse MRGPRs by expressing each of the receptors in human embryonic kidney (HEK) 293 cell and monitoring intracellular calcium changes upon bilirubin application (►**Fig. 2**). They found that only MRGPRA1 could be activated by bilirubin. Since the human MRGPRX family is the closest homology in sequence to the mouse MRGPRA family, they then tested bilirubin against human MRGPRX members and found that bilirubin could also activate MRGPRX4. In the following study, they screened MRGPRX4 and MRGRPA1 against additional bile metabolites using the same calcium imaging assay and showed that bile acids could also activate human MRGPRX4 but not mouse MRGPRA1.[31] Together, their results showed that mMRGPRA1 is a bilirubin receptor whereas hMRGPRX4 is a receptor for both bilirubin and bile acids.

We tackled the cholestatic itch problem using a different strategy.[32] Based on the etiology of cholestasis, we reasoned that the pruritogens of cholestatic itch must exist in bile and that the itch receptors are most likely GPCRs expressed in human DRG neurons. Through bioinformatic analysis using published human transcriptome databases, we selected seven human DRG-enriched nonolfactory orphan GPCRs. We developed a cell line-based TGFα (transforming growth factor-α) shedding assay[39] in HEK 293 cells for detecting the activation of G_s- and G_q-coupled GPCRs (►**Fig. 2**). By expressing the candidate receptors in cells and screening bile extracts, we found that MRGPRX4 was the only GPCR to be activated by metabolites in bile. To further identify active components in the bile, we combined biochemistry methods including HPLC fractionation, mass spectrometry, and nuclear magnetic resonance to identify deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) as the key components in the bile that activate MRGPRX4. We also tested other major bile acids and their derivatives in the human bile and found most of them could activate MRGPRX4. We further screened

Table 2 MRGPR ligands and functions

	Ligand(s)				Proposed functions
Hs. MRGPRX1	CQ Liu et al, 2009[44]	BAM8-22 Lembo et al, 2002[42]	Mucunain Reddy et al, 2018[48]		Itch reaction to an anti-malarial drug; Cowhage-induced itch Cholestatic itch
Mm. MrgprA3					
Mm. MrgprC11		BAM8-22 Han et al, 2002[49] Sanjel et al, 2019[28]	Cathepsin S Reddy et al, 2015[50]	SLIGRL Liu et al, 2011[51]	
Hs. MRGPRX2	Basic secretagogues, Therapeutic drugs Meixiong et al, 2019[43]	Staphylococcus δ-toxin vancomycin Azimi et al, 2017[52]	Mucunain Reddy et al, 2018[48]	SLIGKV Liu et al, 2011[51]	Mast cell degranulation-induced itch; Cowhage-induced itch
Mm. MrgprB2					
Hs. MRGPRX4	Bile acids Meixiong et al, 2019c[38] Yu et al, 2019[39]	Bilirubin Meixiong et al, 2019b[37] Yu et al, 2019[39]			Cholestatic pruritus
Mm, MrgprA1			FMRF Dong et al, 2001[41]	Substance P Azimi et al, 2017[27]	
Hs. MRGPRD	β-alanine Shinohara et al, 2004[53] Liu et al, 2012[45]		Allantoin Yang et al, 2020[54]		β-alanine (an exercise supplement)-induced itch; chronic kidney disease-associated
Mm. MrgprD					

Abbreviation: MRGPR, Mas-related G protein-coupled receptor.

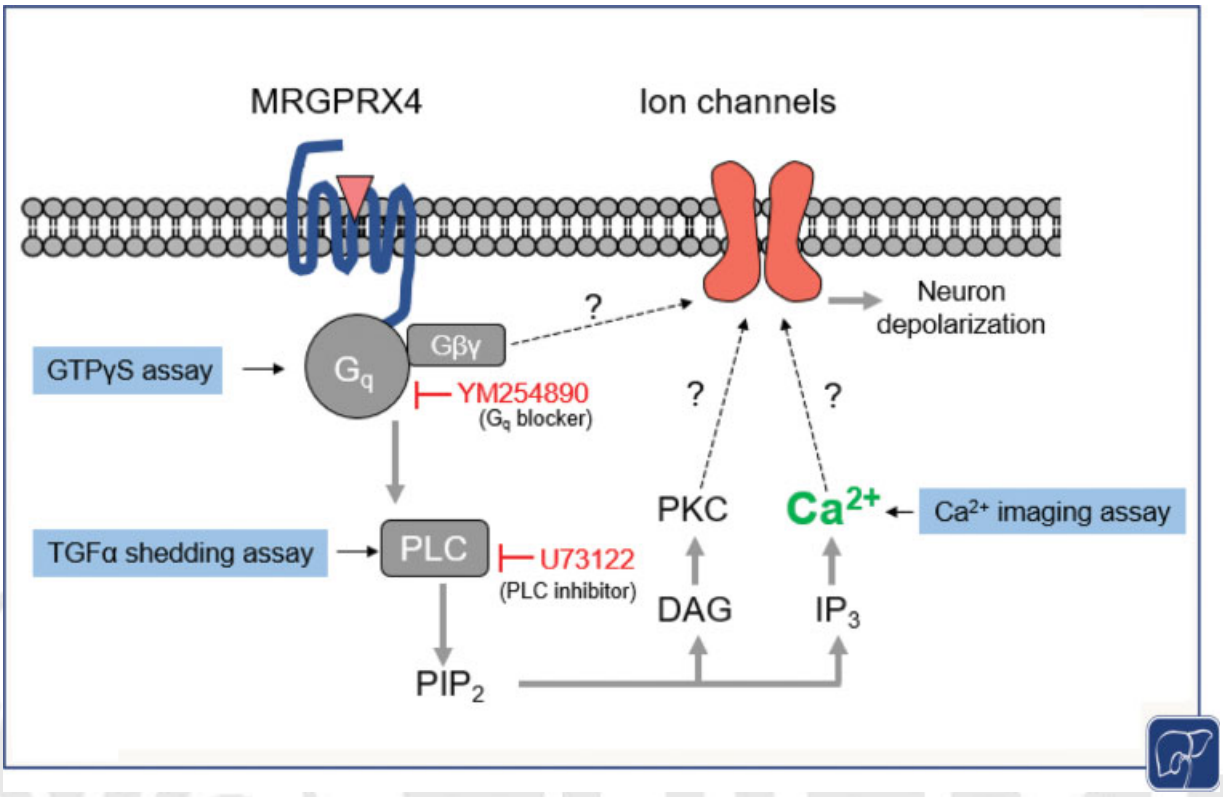

Fig. 2 MRGPRX4 signaling pathway and the assays for its activation. These assays and pharmacological blockers indicate that MRGPRX4 couples with G_q-PLC pathway. The coupling of downstream ion channels with MRGPRX4 to depolarize sensory neurons is currently unclear. DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; MRGPRX4, Mas-related G protein-coupled receptor X4; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase c.

other human, rat, and mouse MRGPRs but found that human MRGPRX4 was the only MRGPR activated by bile acids.

The MRGPRX4 Downstream Signaling

MRGRPX4 is a class A GPCR, which could signal through G_α, G_i, G_q, or $G_{12/13}$.[40] Several lines of evidence suggest that MRGRPX4 mainly signals through the G_q pathway. First, Yu et al detected MRGPRX4 activation by bile in a G_q but not G_s-coupled activation assay[32]; Meixiong et al confirmed that MRGPRX4 couples with G protein using GTPγS assay[30,31]; and Kroeze reported MRGPRX4 activation by K_{ATP}-channel blocker nateglinide in phosphatidylinositol hydrolysis assays[41] (►**Fig. 2**). Second, bile acids and bilirubin elicit calcium increase in MRGPRX4-expressing HEK cells, dissociated mouse DRG neurons, and MRGRPX4-transfected rat DRG neurons, suggesting that MRGPRX4 may couple with the G_q downstream pathway. Moreover, both groups found that pretreatment of MRGPRX4-expressing cells with the G_q blocker YM254890 or the phospholipase c (PLC) inhibitor U73122 abolished bile acid-induced activation (►**Fig. 2**). In sum, these data support that activated MRGPRX4 most likely signals through a G_q-PLC pathway.

MRGPRX4 as a Receptor for Cholestatic Itch

After identifying that human MRGPRX4 is a bile acid and bilirubin receptor and mouse MRGPRA1 is a bilirubin receptor, both groups performed a series of experiments to demonstrate the involvement of MRGPRX4 and MRGPRA1 in cholestatic itch.[30–32] Meixiong et al generated an *MRGPRA1-Cre* transgenic mouse line and an *MrgprA1* null allele to genetically label *MRGPRA1*-expressing cells and study MRGPRA1 functions. They showed that *MRGPRA1* is expressed in a small percentage of adult mouse DRG and trigeminal ganglia sensory neurons that innervate the skin and project to spinal cord lamina I and II, which are major layers receiving itch input from the peripheral nervous system. They also showed that bilirubin activates dissociated mouse sensory DRG neurons using calcium imaging, and that this activation is largely reduced when *MRGPRA1* is genetically ablated. Interestingly, most of these bilirubin-activated neurons are chloroquine-sensitive, indicating that *MrgrpA1* is expressed in a population of itch-related sensory neurons. In addition, they demonstrated that subcutaneous injection of bilirubin-induced scratching in mice was significantly reduced in *MRGPR* cluster (including *MRGPRA1*) and *MRGPRA1*-specific knockout mouse lines. Moreover, in two mouse cholestatic models (induced by α-naphthyl isothiocyanate [ANIT] and cyclosporin,[42,43] respectively), the spontaneous itch is significantly reduced in *MRGPR* cluster knock out and *MRGPRA1* knockout mouse lines. This phenotype was also observed in knockout mice of biliverdin reductase (*Bvr*), a key biosynthetic enzyme for bilirubin.[44] Lastly, pharmacological antagonization of MRGPRA1 by a tripeptide glutaminyl-D-tryptophylphenylalanine (QWF), which is a NK-1R

antagonist but also antagonizes MRGPRA1,[45,46] blocked bilirubin activation of MRGPRA1 and mouse scratching induced by cholestasis. Together, their results provide compelling evidence that bilirubin and MRGPRA1 contribute to cholestatic itch in mice.

In a second study, Meixiong et al generated a humanized mouse line by expressing MRGPRX4 in the MRGPRA3+ itch-selective DRG neuron population.[31] In these transgenic mice, the percentage of bile-acid responsive DRG neurons is significantly increased compared with control wild type mice. These mice also scratch more when challenged intradermally with bile acids and in the cholestatic disease model induced by ANIT.[42] These data suggest that the bile acid-MRGPRX4 pair may also contribute to cholestatic itch in human.

Yu et al demonstrated that MRGPRX4 might mediate cholestatic itch using different methods.[32] After identifying MRGPRX4 as a bile acid receptor, we first tested whether bile acids could function as pruritogens in human. Indeed, intradermal injection of bile acids induced histamine-independent itch in healthy human subjects. This is consistent with the clinical observation that antihistamine drugs are largely ineffective for cholestatic itch. To further demonstrate that the activation of MRGPRX4 is sufficient to induce itch in human, we used a nonbile acid MRGPRX4 agonist nateglinide, which is a K_{ATP}-channel blocker for the treatment of type 2 diabetes but also activates MRGPRX4.[41] Similar to bile acids, nateglinide also triggered itch in healthy human subjects, further supporting the role of MRGPRX4 as an itch receptor in human. At the cellular level, we showed that bile acids could activate a subset of (approximately 5%) primarily cultured human DRG neurons. Consistently, we found that MRGPRX4 is expressed in 6 to 7% of human DRG neurons and that MRGPRX4 co-expresses with histamine receptor H1 (HRH1), an itch neuron marker, using immunostaining and RNAscope in situ hybridization. Taken together, our data indicate that bile acids directly activate itch-related, MRGPRX4-expressing human DRG neurons to induce itch sensation.

Moreover, Yu et al found a positive correlation between plasma bile acid or bilirubin levels and itch intensity in patients with liver diseases.[32] The total bile acid or bilirubin level is significantly higher in itch patients compared with nonitch patients with liver disease or itch patients from skin disorders. We also showed that the averaged plasma bile acid level in cholestatic conditions is sufficient to activate MRGPRX4. Overall, our data provide strong evidence that bile acids/bilirubin and MRGPRX4 may be a critical ligand/receptor pair for mediating cholestatic itch in human.

Potential Species Differences in Molecular Mechanisms of Cholestatic Itch

During the study, Yu et al also found interesting interspecies differences in molecular mechanisms mediating cholestatic itch between human and mice. TGR5, another membrane bile acid receptor, has previously been proposed to mediate cholestatic itch.[15,16] Mouse TGR5 is highly expressed in a subset of DRG neurons, and bile acid activation of mouse DRG neurons was greatly reduced in the absence of *Tgr5*. In addition, the bile acid-induced scratching behavior was significantly reduced in *Tgr5* knockout mice but significantly increased in *Tgr5* overexpressing mice. However, a recent study showed that administering TGR5-selective agonists failed to elicit itch responses in mouse models of cholestasis,[47] and recent clinical trials using TGR5-specific agonists to treat diabetes have not reported itch-related side effects.[48] These data raised questions about TGR5 function in cholestatic itch. Consistent with the reported negative results, we found that intradermal injection of a potent nonbile acid TGR5 agonist, compound 15, did not induce itch in healthy human subjects.[32] When examining the expression pattern of TGR5 in human, monkey, and mouse DRG tissues using both immunostaining and in situ hybridization, we discovered an interesting distinctive expression pattern between primates and mice. TGR5 is expressed in a subset of mouse DRG neurons but only in human and monkey satellite glial cells surrounding sensory neurons. This expression pattern difference fits well with our human psychophysics results. Interestingly, we along with the Dong group tested bile acid activation (including DCA and lithocholic acid) against 12 closely related mouse MRGPRs and found that none of these receptors are activated by bile acids.[30–32] The same is true for seven closely related rat MRGPRs we tested.[32] Together, these results suggest that the primate somatosensory system uses MRGRPX4 whereas the rodent system uses TGR5 for sensing bile acids. Bilirubin, another metabolite that contributes to cholestatic itch, activates both human and mouse MRGPR members.[30,32] However, unlike MRGPRX4, which serves as a convergent receptor for both bile acid and bilirubin receptor, mouse MRGPRA1 can only be activated by bilirubin.

In short, the molecular mechanisms in mediating cholestatic itch seem to be quite different between human and mice. Itch induced by bile acids and bilirubin is mediated by TGR5 and MRGPRA1, respectively, in mice, while MRGPRX4 senses both in human. These inter-species differences must be taken into consideration when developing and testing translational strategies in the future.

Future Directions in MRGPRX4 and Cholestatic Itch Research

Now that we have identified MRGPRX4 as a bile acid/bilirubin receptor mediating cholestatic itch, we can start to address many other interesting, unanswered questions with implications for clinical therapy.

Structural and Functional Studies and Downstream Signaling of MRGPRX4

When examining the structures of bile acids and their potency to activate MRGPRX4, we found that some of the key chemical groups in bile acids are critical for the ligand–receptor interaction. For example, the position of hydroxyl groups in the four-ring core structure and the conjugation of side-chain carboxyl group affect MRGPRX4 signaling activity (►**Fig. 3**). It is still not clear which residues in MRGPRX4 are important for the ligand–receptor interaction though. Future

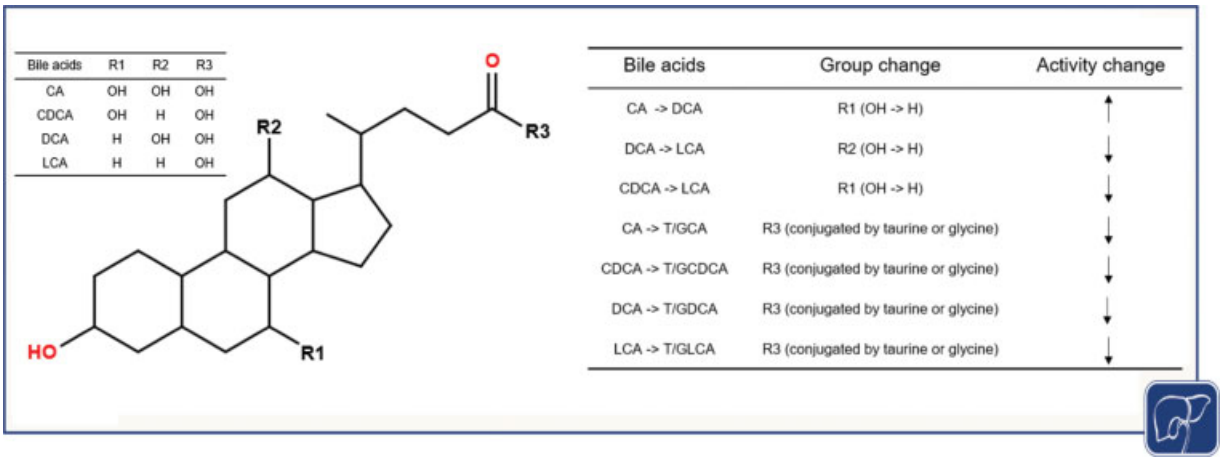

Fig. 3 Bile acids structure and their activity in MRGPRX4 signaling. The general structure of the bile acid consists of a steroid structure with four rings, a five-carbon side chain, and several hydroxyl groups. The inset table above the structure shows the hydroxyl group or carboxyl group of four major bile acids in R1, R2, and R3. The right table shows the activity changes for different bile acids when the R1 or R2 hydroxyl group changes or R3 carboxyl groups are conjugated by taurine or glycine. CA, cholic acid, CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; MRGPRX4, Mas-related G protein-coupled receptor X4; T/G, taurine/glycine.

experiments, such as mutagenesis screening of MRGPRX4 to identify the key residues for bile acids binding or resolving the crystal structure of MRGPRX4 with bile acids will help to answer this question.

Both bile acids and bilirubin activate MRGPRX4. Based on the Fluorescence Imaging Plate Reader calcium imaging assay, we found that bilirubin is 10-time less potent than bile acids, and the maximum activation induced by bilirubin is only 20% of the level induced by bile acids.[32] Given the different structures of bile acids and bilirubin, we tested whether bilirubin is an allosteric modulator of MRGPRX4. Bilirubin can potentiate the activation of MRGPRX4 by bile acids,[32] suggesting that bile acids and bilirubin cooperate in activating MRGPRX4 by binding different parts of the receptor. Mutagenesis screening and resolving crystal structure for MRGPRX4 are needed to help identify the key residues for bilirubin binding. In short, structural and functional analysis of MRGPRX4 and its known ligands will help to not only answer basic biological questions but also to identify additional endogenous and exogenous ligands/antagonists for managing cholestatic itch.

Though studies from both groups showed that activation of MRGPRX4 can induce calcium influx or even action potentials, it is not currently clear which channel(s) function downstream of MRGPRX4 to depolarize primary afferents (►**Fig. 2**). These downstream channels could also be important molecular targets for treating cholestatic itch. Some previous studies suggest that mouse HRH1 and MRGPRA3 couple with transient receptor potential cation channel subfamily V member 1 (TRPV1) and TRPA1 to induce neuron activation, respectively,[49,50] and Meixiong et al showed that the activation of mouse DRG neurons by bilirubin is blocked by applying ruthenium red, which is a nonspecific blocker for TRP and other Ca^{2+} channels.[30] Our RNAscope in situ hybridization revealed that *MRGPRX4* is expressed in a subpopulation of TRPV1 and HRH1 double positive small diameter human DRG neurons.[32] Thus, a worthy hypothesis is that MRGPR4 couples to TRP channels to generate action potential in human DRG neurons. Future experiments are required to determine the exact downstream coupling channels in human DRG neurons with activated MRGPRX4 signaling pathways.

Correlation between Itch Intensity and Bile Acid or Bilirubin Level: A Question to Be Resolved

Despite the compelling evidence provided by both groups, the extent of the contribution of pruritogenic bile acids and bilirubin and their receptor MRGPRX4 to cholestatic itch in human patients needs further careful examination. It has been well documented,[28,51] and we also found, that some patients with high plasma bile acid level do not experience obvious itch while some patients with low levels of plasma bile acids still suffer from severe itch. There are multiple potential reasons to explain the discrepancies that are worthy of in-depth investigation, as we discuss below.

Single-nucleotide polymorphisms (SNPs) of *MRGPRX4* may affect its expression level as well as function. For reasons not currently understood, *MRGPRX4* displays an exceptionally high level of polymorphism. From the gnomAD database, a database containing whole exome sequences from more than 100,000 unrelated individuals from various sequencing projects worldwide,[52] 37.2% of the *MRGPRX4* coding region harbors SNPs. Among all of the missense SNPs, four of them have an allele frequency of greater than 20% (►**Fig. 4**). It remains to be tested whether these SNPs alter the expression level, plasma membrane/subcellular location, affinity to bind to bile acids, or the downstream activation of MRGPRX4. In addition, SNPs in the noncoding region, including the 5′UTR and 3′UTR, could affect the expression level of MRGPRX4. Null, hypo-, or hyperactive alleles of *MRGRPX4* caused by different SNPs could potentially explain some discrepancy in the itch symptom.

The exact bile acid constitution may also affect the cholestatic itch symptom. Cholic acid (CA) and CDCA are the major primary bile acids synthesized in human livers.[53] DCA and lithocholic acid (LCA) are secondary bile acids

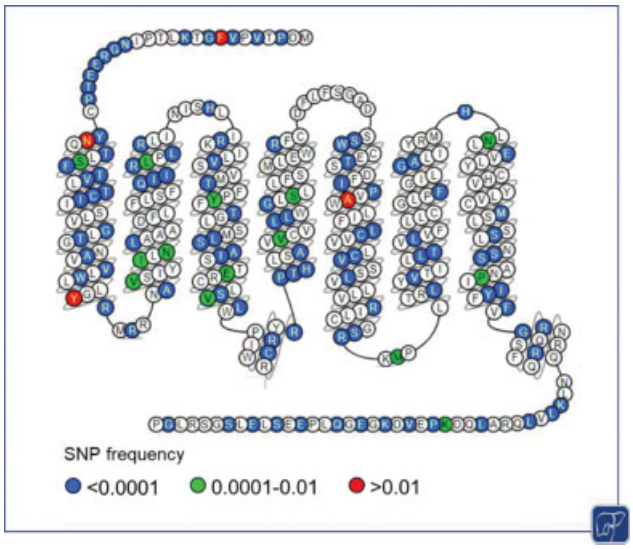

Fig. 4 SNPs in human MRGPRX4. The two-dimensional structure of MRGPRX4. The letters in the small circles represent each amino acid. The colored circles show the SNPs identified in the gnomAD database that change the amino acid residues in the protein (*blue*, allele frequency < 0.0001; *green*, allele frequency between 0.0001 and 0.01; *red*, allele frequency > 0.01). MRGPRX4, Mas-related G protein-coupled receptor X4; SNP, single-nucleotide polymorphism.

produced in the intestine.[54] We have showed that different bile acids activate MRGPRX4 with different efficacies and potencies. For example, the DCA is 50 to 100-fold more potent than conjugated primary bile acids. Gut microbiota plays a critical role in regulating bile acid synthesis and influences the constitution of bile acid species in different individuals.[55–57] For example, the activity of cholesterol 7α-hydroxylase, the rate limiting enzymes in bile acid synthesis, is modulated by the composition of gut bacteria[56]; bacterial 7α-dehydroxylase converts CA and CDCA to DCA and LCA in the distal intestine.[58] Thus, the bile acid metabolism and gut microbiota could significantly affect the bile acid species in plasma or skin and contribute to interpatient differences in pruritus intensity. A detailed profiling and quantification of individual bile acids rather than total bile acids would better evaluate the role of bile acids and MRGPRX4 in cholestatic itch. Manipulation of gut microbiota, which might be an effective strategy to manage cholestatic itch, should be further studied.

In addition to bile acids, bilirubin is another endogenous agonist for MRGPRX4. Despite the long-standing association between jaundice and itch, bilirubin itself has not been considered as a pruritogen due to some clinical observations. For example, itch may precede the appearance of jaundice and there is often a significant relief of itch before a fall in plasma bilirubin,[59] and the hyperbilirubinemia and jaundice that occur in hemolytic anemia are not associated with pruritus.[60] As discussed above, bilirubin has a lower potency and efficacy to activate MRGPRX4 compared with bile acids.[30–32] Thus, bilirubin alone, even in a relatively high concentration, may not reach to the threshold to activate the itch sensation through MRGPRX4, which may explain why there is a poor correlation between bilirubin level and itch symptom. We propose that alleviation of both bilirubin and bile acids would better predict incidence of cholestatic itch than bilirubin alone.

To activate itch-related nerve fibers, bile acids and bilirubin must accumulate in proximity to MRGPRX4, such as at the skin or sensory neuron cell bodies (►**Fig. 5**). At present, it is unclear whether deposition of bile acids and bilirubin in the skin and/or DRG is a process of passive diffusion or an actively controlled process involving particular transporters. Several bile acid transporters have been identified, such as

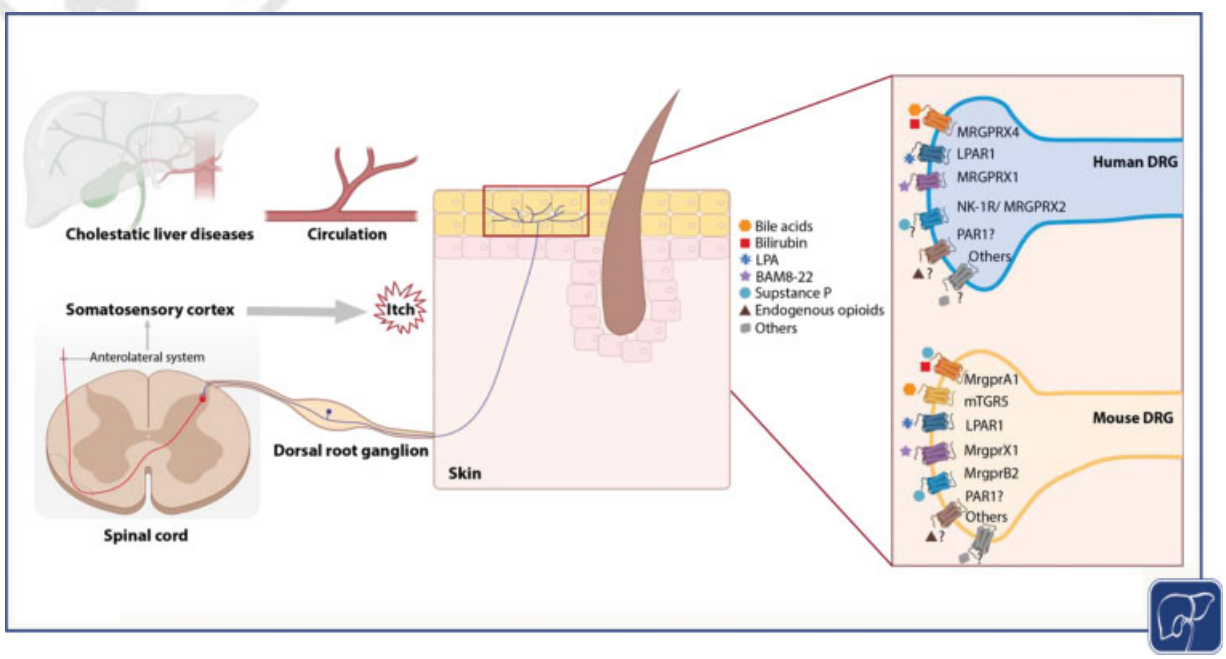

Fig. 5 Summary of current molecular mechanisms proposed for cholestatic itch. In the cholestatic conditions, the itch causing molecules are accumulated in the liver and spill out into circulation. The pruritogens then are accumulated in the skin and activate itch-related sensor fibers through their receptors. The itch signals are then relayed from spinal cord to the brain and generate itch sensation.

bile acids export pump, multidrug resistance protein 2, Na^+-dependent apical sodium-dependent bile acid transporter, organic solute transporter α and β, Na^+-dependent taurocholic co-transporting polypeptide (NTCP), and organic anion transporting polypeptides.[61] If some of these transporters are involved in transporting bile acids during their metabolism or from the locations of synthesis to the locations of triggering itch sensation, polymorphisms in these genes and their expression levels may also affect the itch symptom. Indeed, studies have shown that in patients with NTCP mutations there are very high levels of total bile acids in the serum, but none of the patients had pruritus.[62,63] This intriguing phenomenon will need further studies to understand the underlying mechanisms. Systematic genome-wide association studies of patients with cholestatic itch could provide more insight into whether these transporters participate in cholestatic itch.

Moreover, other compounds may also activate MRGPRX4 and thus be involved in cholestatic itch. For an example, during the ICP, most of the patients with itch symptoms have a low plasma bile acid level.[64] Since female hormones could reach up to millimolar during the pregnancy,[65] and since female hormones have the similar chemical structures to bile acids (both are steroids), it worth testing whether these female hormones could activate MRGPRX4 and induce itch in pregnancy. One striking fact is that the known agonists for MRGPRX4, including bile acids, bilirubin, and nateglinide, all have different structures. Thus, compounds with different structure could still be ligands of MRGPRX4. Lansu et al recently identified 54 MRGPRX4 agonists after screening a 5,695-compound library,[66] suggesting an expansive role played by MRGPRX4 in physiology and pathology.

Last but not least, other metabolites and receptors may also participate in cholestatic itch. Though TGR5 is expressed in human satellite glia cells surrounding the DRG neurons and does not seem to contribute to acute itch, it remains to be determined whether DRG neuron activities will be influenced by TGR5+ glial cells when bile acid level is chronically increased in patients with cholestasis. For other examples, LPA and its synthetase autotaxin (ATX) have been reported to correlate with cholestatic itch[17]; BAM8–22 and its receptor MRGPRX1 (also called MRGPRC11 in mice) have been implicated in cholestatic itch in mice.[21] In addition, endogenous opioids, histamine, serotonin, progesterone, and estrogens have also been proposed as pruritogens for cholestatic itch. Both supportive and contradictory results have emerged from studies on candidate pruritogens in cholestatic itch.[13] Thus, key evidence, such as whether these metabolites can induce itch in human, whether their corresponding receptors are expressed in itch-related neurons, and whether these metabolites increase in the cholestatic itch patients, remain to be seen and may clarify the causal relationship between these compounds and cholestatic itch.

Conclusion

Cholestatic itch is a common disturbing symptom in chronic liver diseases. Several metabolites have been proposed as pruritogens for cholestatic itch, but no single hypothesis can explain all the cases, reflecting the complex etiology of cholestatic itch. The recently identified bile acid and bilirubin receptor MRGPRX4 provides a novel molecular target and has opened a new avenue for understanding the etiology of cholestatic itch. Though future experiments are needed to further clarify structure and function MRGPRX4, it is a promising drug target for developing therapies to treat itch in cholestasis.

Main Concepts and Learning Points

- Human MRGPRX4 is a receptor for bile acids and bilirubin.
- MRGPRX4 is a primate-specific gene and contributes to cholestatic itch in human.
- There are species differences in molecular mechanisms in mediating cholestatic itch between human and rodents.
- MRGPRX4 is a promising target for developing anti-itch therapeutics.

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Conflict of Interest
None declared.

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