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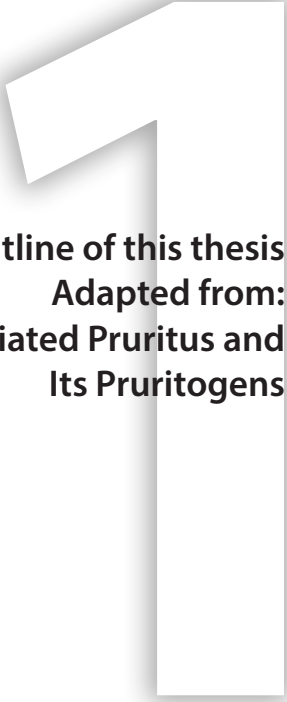
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General introduction and outline of this thesis
Adapted from:
Cholestasis-Associated Pruritus and
Its Pruritogens

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Abstract

Pruritus is a debilitating symptom of various cholestatic disorders, including primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and inherited progressive familial intrahepatic cholestasis (PFIC). The molecular mechanisms leading to cholestasis-associated pruritus are still unresolved and the involved pruritogens are indecisive. As a consequence of pruritus, patients suffer from sleep deprivation, loss of daytime concentration, auto-mutilation and sometimes even suicidal ideations. Current guideline-approved therapy of cholestasis-associated pruritus includes stepwise administration of several medications, which may alleviate complaints in some, but not all affected patients. Therefore, also experimental therapeutic approaches are required to improve patients' quality of life. This article reviews the current state of research on pruritogens and their receptors, and shortly discusses the most recent experimental therapies.

Introduction

Cholestasis is the term for diminished or impaired bile flow generated by hepatocytes and cholangiocytes. The cause of cholestasis can be intra- or extrahepatic, and can be genetic or the consequence of an inflammatory or malignant hepatobiliary disease. Next to fatigue, pruritus is the most frequent symptom in patients with chronic cholestatic disorders and may affect more than half of patients with fibrosing cholangiopathies such as primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) at least transiently during their disease course. Depending on the cause of cholestasis, 30–90% of patients suffer from chronic pruritus, which is unresponsive to antihistamines. Chronic pruritus can lead to loss of concentration, sleep deprivation and automutilation or prurigo nodularis due to scratching (Beuers et al., 2014). In most serious cases, suicidal ideations may occur and the burden of pruritus can become the primary indication for liver transplantation. Cholestasis-associated pruritus shows a diurnal rhythm with increased intensity in the late evening and early night. The itch is typically localized at the limbs and soles of the feet and at the forearms and palms of the hands, but may also be generalized.

Liver diseases over a wide range of prevalences are associated with chronic pruritus including PBC, PSC and secondary sclerosing cholangitis (SSC), cholangiocarcinoma, viral hepatitis, drug-induced cholestasis, sarcoidosis hepatitis, and rare inherited forms of cholestasis. For intrahepatic cholestasis of pregnancy (ICP) pruritus is one of the mandatory diagnostic criteria and is observed in 0.5–2.0% of pregnant women, PBC is found in about 0.1% of women at an age of 50 years, whereas Alagille syndrome as an example of rare genetic diseases is found in only one of 100,000 children and adolescents. In some cases, such as PBC, pruritus can be the first symptom of the disease. Since this is a relatively rare disease, diagnosis by general practitioners or dermatologists may be difficult.

It has not been established whether environmental, geographical or life-style factors influence the prevalence or severity of cholestasis-associated itch. However, for ICP there are indications for possible environmental factors that influence the occurrence of ICP in genetically susceptible individuals. It has been postulated that the long-chain monounsaturated fatty acid erucic acid and/or selenium may play a role. Furthermore, the occurrence of ICP was exceptionally high in Chile a number of decades ago, but has drastically decreased more recently, suggesting that a change in diet or unknown lifestyle factor might play a role in ICP (Arrese et al., 2008; Reyes, 2016).

The pathogenesis of cholestasis-associated pruritus is still poorly understood, however, based on research of the past 40 years and therapies that give relief of pruritus in a subset of patients, there is common understanding that:

- » Potential pruritogens are located in the systemic circulation, as indicated by (partial) relief of pruritus after treatment with plasmapheresis, albumin dialysis or plasma separation/anion absorption.
- » Direct or indirect pruritogens are excreted into bile and undergo enterohepatic circulation, as suggested by attenuation of pruritus in some patients after oral

administration of anion exchange resins, and by inhibitors of the apical sodium-dependent bile acid transporter (ASBT) or effectively, but only transiently by nasobiliary drainage.

- » Pruritogens are thought to be formed or biotransformed in the liver and/or the gut as indicated by effective treatment with the potent pregnane X receptor (PXR) agonist, rifampicin.
- » Pruritogens affect the endogenous opioidergic and serotonergic system, as suggested by antipruritic activity of opioid antagonists such as naltrexone and serotonin reuptake inhibitors such as sertraline.

In this review, we will discuss the current literature on cholestasis-associated pruritus, with a specific disquisition of the possible pruritogens. We will briefly deliberate the receptors involved in pruritus and current treatment options.

Itch Signaling

Signaling of itch is often measured after provocation with histamine, chloroquine or cowhage, which activate the histamine 1 (H1R) or 4 (H4R) receptor (Bell et al., 2004), the Mas-related G-protein X1 in humans (MRGPRX1) or A3 in mice (MRGPRA3) (Liu et al., 2009), and the protease-activated receptor 2 (PAR2) or 4 (PAR4) (Reddy et al., 2008), respectively.

Histamine-induced itch signaling occurs through mechano-insensitive C-fibers (CMi) with unmyelinated nerve endings in the skin (Andrew and Craig, 2001; Schmelz et al., 1997). However, the majority of chronic itch types including cholestasis-associated pruritus cannot be relieved by anti-histamine treatment, and are therefore classified as non-histaminergic itch (Andersen et al., 2017; Davidson et al., 2007; Shelley and Arthur, 1955a; Shelley and Arthur, 1955b; Yosipovitch et al., 2018). Non-histaminergic itch in humans possibly signals through mechano-heat-sensitive C-fibers (CMH), as measured with cowhage provocation (Johanek et al., 2008; Namer et al., 2008).

Itch signals run from primary itch neurons in the skin through the dorsal root ganglia (DRG) to a secondary neuron in the dorsal horn of the spinal cord (Mishra and Hoon, 2013) with natriuretic peptide B (NPPB) and glutamate as the neurotransmitters (Koga et al., 2011; Lagerstrom et al., 2010; Liu et al., 2010; Mishra and Hoon, 2013). In the dorsal horn of the spinal cord, the secondary neuron crosses to the contralateral side and transmits the signal by gastrin-releasing peptide (GRP) to a tertiary neuron (Mishra and Hoon, 2013; Sun and Chen, 2007; Sun et al., 2009). This third neuron projects through the spinothalamic tract to the ventromedial nucleus of the thalamus.

In order to reduce itch, the central nervous system provokes an urge to create a local pain signal by scratching. This demonstrates that pain has an inhibitory effect on itch (Ikoma et al., 2006; Yosipovitch and Bernhard, 2013). When pain is pharmacologically reduced, for example by morphine administration, itch frequently sets in at the region of reduced pain transmission (Atanassoff et al., 1999; Ballantyne et al., 1988). This contra-mechanism is very

likely fulfilled by inhibitory interneurons in the dorsal horn of the spinal cord, which create a constant tonus of inhibition by nociceptive to pruriceptive neurons. In genetic experiments with mice, it was shown that when these inhibitory interneurons are not formed, due to deletion of the gene for the transcription factor *Bhlhb5*, mice suffer from severe chronic itch (Ross et al., 2010).

Many receptors that are involved in pruritus are G-protein coupled receptors, which, after activation, cause an increase in intracellular calcium release and thereby activate PLC and PKC [reviewed by (Dong and Dong, 2018)]. PLC and PKC are often coupled to TRP-channels that amplify the intracellular cation wave and together with NaV channels initiate an action potential, leading to itch sensation (Amadesi et al., 2006; Imamachi et al., 2009; Jurcakova et al., 2018; Wilson et al., 2011). Next to their role in coupled cation influx, these TRP-channels can also be activated directly by chemical, thermal and mechanical noxious stimuli (Kittaka and Tominaga, 2017; Moore et al., 2018; Xie and Hu, 2018).

Pruritogens That Are Potentially Involved in Cholestasis-associated Itch *Bile Salts*

The most abundant components of bile are bile salts, which for a long time were, and still are thought by some, to act as pruritogens in cholestasis. There are several subspecies of bile salts with different properties. They can bind to the intracellular farnesoid X receptor (FXR) which is a nuclear receptor that regulates a considerable transcription network, and to the transmembrane G protein-coupled receptor TGR5 that upon activation induces cAMP synthesis. A specific subset of bile salts can even cause a response through the pregnane X receptor, vitamin D receptor and constitutive androstane receptor, thereby influencing bile salt synthesis and progression of cholestasis (Schaap et al., 2014).

Bile salt levels are elevated in serum of cholestatic patients (Trottier et al., 2012; Woolbright et al., 2015). Elimination of bile with all its substances by nasobiliary drainage, or removal of albumin-bound substances (including bile salts) by albumin dialysis, often cause a tremendous diminution in the perception of itch, although this only lasts for a few weeks to months after the intervention (Beuers et al., 2006; Hegade et al., 2016; Pares et al., 2010; Stapelbroek et al., 2006). The results of these therapies clearly indicate that pruritogen(s) or progenitors of pruritogens are excreted in bile, but not necessarily that bile salts are causing cholestasis-associated pruritus.

Early research aimed at binding bile salts in the gut by using anion exchange resins (and thereby also bile salt sequestrants) like cholestyramine, which resulted in some reduction of pruritus (Carey, 1961; Datta and Sherlock, 1966; Oster et al., 1965). However, a more recent report indicates that the later developed, more potent anion exchange resin colesevelam does not improve pruritus more than placebo, despite the fact that bile salt levels were reduced by nearly 50% (Kuiper et al., 2010).

The group of Bunnett intensely studied itch signaling by bile salts. They proposed that itch signaling is mediated by the bile salt receptor TGR5. *Tgr5* expression was detected in

the soma of DRG neurons of mice (but not in nerve fibers in the skin or the dorsal horn). Activation of DRG neurons led to the release of the neuropeptide gastrin-releasing peptide (GRP) and the opioid peptide leucine-enkephalin, which are thought to be itch and analgesia transmitters (Alemi et al., 2013). A year later, the same group showed that the TGR5 receptor was co-expressed with the itch channel TRPA1 and activation of this axis induced scratch activity in mice (Lieu et al., 2014). Indications for the involvement of TGR5 in itch signaling seem strong, but the role for bile salts as candidate pruritogens in relation to cholestasis-associated pruritus remains inconclusive. Several clinical studies have shown that there is no correlation between bile salts in serum, urine or skin and the intensity of pruritus (Bartholomew et al., 1982; Freedman et al., 1981; Ghent et al., 1977; Kremer et al., 2010). Pruritus is often one of the first symptoms of cholestatic liver disease, while bile salts levels are still relatively low (Geenes and Williamson, 2009; Kremer et al., 2008). During late stages of the disease, or in obstructive cholestasis, when bile salt levels can increase to high concentrations, pruritus is sometimes reported to subside (Murphy et al., 1972).

Another receptor that is postulated to be involved in bile salt-induced itch signaling is the mas-related G protein receptor X4 (MRGPRX4). Mouse MRGPR orthologs were not activated by bile salts and therefore the human receptor was expressed in mice and scratch activity was reported to be increased upon subcutaneous injection of the pruritogen chloroquine and the bile salt deoxycholate (DC) (Meixiong et al., 2019b). However, injection with unconjugated bile salts is not very representative for cholestasis, since conjugated primary bile salts [like taurochenodeoxycholate (TCDC) and glycochenodeoxycholate (GCDC)] are increased during cholestasis whereas the pool size of conjugates of the secondary bile salt DC is reduced in cholestasis (Beuers et al., 1992). Moreover, unconjugated bile salts diffuse into cells and cause increases in cytosolic free calcium (Dziki et al., 1995; Lau et al., 2005). In the same report, mice were treated with the hepatotoxicant α -naphthylisothiocyanate (ANIT), which is a known inducer of cholestasis, serious liver damage, including hepatocellular necrosis and liver fibrosis (Golbar et al., 2013; Joshi et al., 2017; Tjandra et al., 2000; Xu et al., 2004). Scratch activity was found to be increased in mice expressing MRGPRX4 compared to control animals, but only during the first 2 days of cholestasis (Meixiong et al., 2019b), suggesting that this signaling undergoes fairly rapid desensitization. Such a short period of scratch behavior is not representative of the human situation, in which pruritus usually lasts for months and years. Moreover, in two other reports ANIT treatment did not lead to increased scratch activity (Cipriani et al., 2015; Langedijk et al., 2021).

A second, independent, report on MRGPRX4 showed that intradermal injection of 500 μ g DC induced an acute itch sensation in healthy volunteers (Yu et al., 2019). These researchers showed that *MRGPRX4* mRNA is expressed in 6–8% of human DRG neurons and co-expressed with H1R, TRPV1, and Nav1.7 voltage-gated sodium channel; which are all established itch-related channels. Since high concentrations of administered bile salts can lead to direct activation and degranulation of mast cells, and thereby release of histamine (Quist et al., 1991), the authors included the administration of antihistamines, which did not reduce DC-induced itch, indicating a non-histaminergic mechanism. However, DC, which showed

to be the most potent ligand for MRGPRX4 among all tested bile salts, was not present in plasma of itchy vs. non-itchy patients with cholestasis. Other bile salts that showed a bigger difference in plasma of itchy vs. non-itchy patients with cholestasis were only weak agonists for MRGPRX4 (Yu et al., 2019).

Together, these results indicate that bile salts are involved in many processes, by binding to several receptors that are, among others, located on sensory neurons. However, as pruritogens for cholestasis-associated itch, plasma bile salt concentrations do not correlate with clinical complaints of itch. The most prominent example against the theory of bile salts as pruritogens is represented by patients suffering from Na⁺-taurocholate cotransporting polypeptide (NTCP)-deficiency. The plasma bile salt levels of these patients can be extremely high, but patients do not complain of pruritus (Erlinger, 2015; Van Herpe et al., 2017; Vaz et al., 2017; Vaz et al., 2015).

In conclusion, human clinical observations overrule all in vivo and in vitro research and pose the strongest argument against bile salts as dominant pruritogens in cholestasis-associated itch.

Endogenous Opioids

The role of endogenous opioids in cholestasis-associated pruritus has recently been reviewed by Bergasa (Bergasa, 2018). Here, we want to discuss the most important arguments that support or dispute the role of endogenous opioids as causative pruritogens in cholestatic liver disease.

The central mechanism of pruritus is thought to include upregulation of the μ -opioid receptor system and suppression of the κ -opioid receptor system (Kamimura et al., 2017). One of the first observations in relation to opioids and pruritus is the fact that the μ -receptor agonist morphine can induce pruritus in humans (Ballantyne et al., 1988; Cousins and Mather, 1984), while the μ -receptor antagonist naloxone can inhibit this reaction (Bergasa et al., 1992; Bernstein et al., 1982). Endogenous opioid levels, including methionine enkephalin, leucine enkephalin and β -endorphin (μ -receptor agonists), were increased in plasma of bile duct ligated (BDL) animals and cholestatic patients with cirrhosis (Spivey et al., 1994; Swain et al., 1992; Thornton and Losowsky, 1988). However, in a more recent study the plasma levels of these μ -receptor agonists were comparable between cholestatic patients with and without pruritus, and were not increased in women with ICP compared to regular pregnancies (Kremer et al., 2010). As was shown before in PBC patients, methionine enkephalin concentrations correlate with the stage of disease but not with the severity of pruritus (Spivey et al., 1994). Most studies into the beneficial effects of opioid antagonists involved few patients (in some cases even case reports) and/or were not placebo-controlled. Although treatment with μ -receptor antagonists like naloxone and naltrexone could reduce the level of pruritus in cholestatic patients, it can also lead to an opiate withdrawal-like reaction (Bergasa et al., 1992; Jones and Dekker, 2000; Mansour-Ghanaei et al., 2006; Shawcross and Jalan, 2001; Tandon et al., 2007; Thornton and Losowsky, 1988).

Next to inhibiting the μ -opioid receptor, studies have been performed on activating the κ -opioid receptor system in mice, thereby reducing scratch activity induced by substance P, histamine or morphine (Togashi et al., 2002; Umeuchi et al., 2003). The κ -receptor agonist nalfurafine has been studied in Japan, to treat pruritus in patients with chronic kidney disease (Kumagai et al., 2010; Wikstrom et al., 2005) and chronic liver disease (Kamimura et al., 2017; Kumada et al., 2017; Yagi et al., 2018). Treatment showed a partial decrease in levels of pruritus without any severe adverse events. The κ -opioid receptor agonist asimadoline is being tested in a phase II clinical trial to assess its efficacy on the relief of pruritus in patients with atopic dermatitis (2017). For an overview on κ -opioid receptor agonists, see (Cowan et al., 2015).

LPA/ATX

We have reported in earlier studies that lysophosphatidic acid (LPA) is a potential pruritogen in cholestasis (Kremer et al., 2016; Kremer et al., 2010) LPA in serum of pregnant women with ICP and in serum of PBC patients with pruritus induced an increased Ca^{2+} response when applied to neuronal cells (Kremer et al., 2010). Intradermal injection of LPA in the skin of mice initiated a scratch response, which confirmed a previous independent study (Hashimoto et al., 2004; Kremer et al., 2010). The main production of LPA comes from the phospholipase D autotaxin (ATX), which catalyzes the hydrolysis of lysophosphatidylcholine (LPC) into LPA and choline (Tanaka et al., 2006; Tokumura et al., 2002; Umezu-Goto et al., 2002). ATX (ENPP2) belongs to the family of ectonucleotide pyrophosphatases/phosphodiesterases (ENPP1-7) and promotes multiple functions like cell migration, angiogenesis and metastasis (Moolenaar and Perrakis, 2011). A direct causal relation between plasma ATX and production of LPA is seen in ATX heterozygous mice, which show a reduction of 50% in ATX activity and plasma LPA levels, while ATX-deficient mice die prematurely due to vascular defects (van Meeteren et al., 2006). High ATX mRNA expression in human tissues has been described for brain, ovary, lung and kidney, while enzyme activity has been detected in blood, cerebrospinal and seminal fluid, urine and saliva [reviewed in (Jankowski, 2011)]. So far, it is still unclear which of these organs contribute to the circulating ATX levels in human plasma and cerebrospinal fluid. Recently, our group has shown that human enteroendocrine cells of the small intestine are also a source of ATX (Bolier et al., 2016).

LPA can activate neuronal cells, satellite glia cells and other cell types via six different LPA-specific receptors (LPAR1-6) (Meyer Zu Heringdorf, 2004; Robering et al., 2019; van Meeteren and Moolenaar, 2007). It has been described that LPA can induce neuropathic pain via LPAR1, LPAR3 and LPAR5 (Choi and Chun, 2013). However, in a study using the cheek injection mouse model Kittaka et al. (Kittaka et al., 2017) showed that LPA is only a mediator of itch rather than of pain. In this study, LPA was found to activate LPAR5, which, via intracellular phospholipase D activation, generates intracellular LPA that can activate TRPA1 and TRPV1, and thereby induce itch sensation.

The crystal structure of ATX shows an active site containing a hydrophilic groove, a hydrophobic lipid-binding pocket and a hydrophobic channel (tunnel) (Hausmann et al.,

2011; Inoue et al., 2011; Nishimasu et al., 2011). This tunnel structure is able to selectively bind steroids and bile salts that lead to inhibition of ATX activity (Keune et al., 2016). The question remains whether more compounds involved in cholestasis can bind in this tunnel, and this way influence ATX activity.

Serum ATX levels are prominently increased in cholestatic patients with pruritus compared to cholestatic patients without pruritus, and in pregnant women with ICP compared to regular pregnancy. In our study, ATX activity correlated significantly with intensity of pruritus and with the effectiveness of treatment by rifampicin, MARS and nasobiliary drainage (Kremer et al., 2016; Kremer et al., 2010). Serum ATX levels also indicate a therapeutic response to treatment with bezafibrate (Kremer et al., 2019), prednisolone (Sumida et al., 2013) and plasmapheresis (Heerkens et al., 2019). Even though ATX is not excreted into bile (Kremer et al., 2010), interruption of the enterohepatic circulation by nasobiliary drainage and ASBT-inhibition still led to a decrease in both circulating ATX levels and pruritus scores (Al-Dury et al., 2018; Hegade et al., 2017; Hegade et al., 2019; Kremer et al., 2010). These findings strongly indicate a tight relation between serum ATX levels and pruritus.

On the other hand, serum ATX levels are similarly increased in (pathological) conditions without itch, including regular pregnancy, some cancer entities and chronic viral hepatitis B and C. In several liver diseases accompanied by liver fibrosis (but not itch), serum ATX levels can also act as a marker of severity of liver injury [including non-alcoholic steatosis hepatitis (NASH) (Fujimori et al., 2018), PBC and PSC (Joshita et al., 2018b; Wunsch et al., 2016), hepatitis B (Joshita et al., 2018a) and hepatitis C (Yamazaki et al., 2017)]. A recent study reported that ATX levels correlate with fibrosis markers but not with frequency and severity of pruritus in PBC patients (Fujino et al., 2019). A paradox seems to remain in the relation of serum ATX levels to pruritus. A common factor might be involved in both pruritus and liver fibrosis and therefore share ATX as a biomarker. Together, these results suggest that research into other cholestatic factors, that play a more dominant role in the initiation and/or potentiation of pruritus in cholestasis, is warranted.

Serotonin

Serotonin (5-HT) is a neurotransmitter in the central nervous system that can directly activate sensory neurons (Beck et al., 1974; Akiyama et al., 2010; Klein et al., 2011). Serotonin can be released from mast cells in the skin and can act as a pruritogen (Kushnir-Sukhov et al., 2007; McNeil and Dong, 2012; Zhao et al., 2014). The serotonergic system might be deregulated in cholestatic patients (Schworer et al., 1995) and in patients with atopic dermatitis (Soga et al., 2007), leading to chronic pruritus. In rats with cholestasis induced by BDL, serotonin levels in the skin and spinal cord were significantly increased compared to sham mice, and enhanced scratching behavior was noted after mechanical and heat stimulation (Tian et al., 2016). In the absence of mechanical and heat stimuli, however, these mice showed no increased spontaneous scratching activity while they were clearly cholestatic.

Seven classes of 5-HT receptors have been identified (5-HT₁₋₇) that comprise at least fifteen subtypes, of which all but one are metabotropic GPCRs (Bockaert et al., 2006; Hoyer

and Martin, 1997). 5-HT₂ receptors are suggested to be important for itch perception (Akiyama et al., 2016; Yamaguchi et al., 1999), by signaling through G_{q/11}, which activates PLC (Bockaert et al., 2006; McNeil and Dong, 2012). Many pruritogens bind to metabotropic receptors on primary sensory neurons and are coupled to ionotropic channels via intracellular signaling pathways to allow sufficient current influx to generate action potentials (Snyder et al., 2016). The cation channels TRPV1 and TRPA1 are often involved in itch transmission (Ross, 2011). A similar mechanism of a metabotropic receptor coupling to an ionotropic channel was found by Morita et al., where serotonin induces itch via activation of 5-HT₇, which was shown to be coupled to TRPA1 (Morita et al., 2015). In a mouse model of atopic dermatitis, where mice were lacking 5-HT₇ or TRPA1, the animals displayed reduced scratching. The authors suggest a role for 5-HT₇ antagonists in the treatment of a variety of pathological itch conditions (Morita et al., 2015). By investigating the peripheral neuronal mechanisms underlying pruritus, Imamachi et al. showed that serotonin-induced pruritus in mice required the presence of PLCβ3 and TRPV1-expressing neurons, although not the TRPV1-channel itself (Imamachi et al., 2009). The TRPV4-channel, however, does seem to be involved as shown by reduced serotonin-evoked scratch bouts in TRPV4 knockout mice and wild type mice treated with a TRPV4 antagonist (Akiyama et al., 2016). The miRNA *mir-203b-3p* induces TRPV4 activation through 5-HT_{2B}, as shown in a mouse model of psoriasis (De Logu et al., 2023). Selective serotonin reuptake inhibitors (SSRIs) have shown some effect against pruritus in cholestatic patients (Browning et al., 2003; Mayo et al., 2007; Stander et al., 2009; Thebaut et al., 2017). They inhibit pre-synaptic reuptake of serotonin and might dull transmission of nociceptive stimuli through unmyelinated C-fibers (Beck and Handwerker, 1974; Sommer, 2004). When standard therapies have no effect on the reduction of pruritus, the SSRI sertraline is advised as a fourth line treatment (EASL, 2009). Notably, chronic pruritus is often associated with psychopathology, including anxiety and depression, where SSRIs may improve pruritus by treating underlying psychiatric comorbidities (Gupta and Gupta, 1999).

Histamine

Histamine is the best established pruritogen during acute allergic reactions, by activating histamine receptors (which are GPCRs) and TRP channels on sensory nerve endings. Antagonists of H1R are useful for the treatment of acute allergic pruritus, but are ineffective in the treatment of cholestasis-associated pruritus (EASL, 2009). Although plasma histamine levels have been reported to be slightly increased in cholestatic disease (Gittlen et al., 1990), in the study of Kremer et al., no correlation was found between plasma histamine levels and itch intensity in pruritic patients with ICP, PBC and other cholestatic disorders (Kremer et al., 2010). Also skin changes consistent with histamine-mediated effects, like axon-reflex erythema (Ikoma et al., 2005), are not seen in cholestatic disease. Sedation with antihistamines may have a non-specific beneficial effect by improving sleep at night, but they reduce concentration during the day (Jones and Bergasa, 2000). Primary afferent neurons, responsible for histamine-induced itch in humans, belong to the group of

mechano-insensitive unmyelinated C-fibers (Ikoma et al., 2006; Schmelz et al., 1997; Schmelz et al., 2003). However, a second group of C-fibers exist, that are insensitive to histamine, but sensitive to low intensity, high frequency electrical stimulation, which could explain itch without accompanying axon-reflex erythema (Ikoma et al., 2005; Ikoma et al., 2006).

Bilirubin

Bilirubin is an essential component in bile and is the direct cause of jaundice in cholestatic diseases. It was recently hypothesized that bilirubin could act as a pruritogen by stimulating human MRGPRX4 and mouse MRGPRA1 (Meixiong et al., 2019a). MRGPRs are a family of GPCRs expressed in DRGs on primary sensory neurons (Dong et al., 2001; Flegel et al., 2015). In the study of Meixiong, the mouse receptor MRGPRA1 and the human receptor MRGPRX4 were expressed in mouse DRGs that lacked expression of any other MRGPR (*Mrgpr* cluster KO mice). Bilirubin could activate 14 and 32% of the respective DRGs. Induction of cholestasis in mice by treatment with the hepatotoxicant ANIT led to hyperbilirubinemia and increased scratch activity, which was reduced in *mrgpra1* KO and biliverdin reductase KO mice. From these results, the authors suggest that high serum bilirubin levels are involved in cholestasis-associated pruritus by binding to MRGPRX4 on sensory neurons (Meixiong et al., 2019a).

Results from clinical observations, however, suggest otherwise. It has been shown multiple times that plasma bilirubin correlates poorly with pruritus intensity in cholestatic patients (Freedman et al., 1981; Kremer et al., 2016). Pruritus is often observed while at the same time serum bilirubin levels are hardly elevated (EASL, 2009). Furthermore, many patients that suffer from pruritus are not jaundiced and vice versa, many jaundiced patients do not complain of itch. Patients with Dubin Johnson syndrome or Crigler Najjar syndrome type I have medium to very high plasma levels of bilirubin (conjugated and unconjugated, respectively) and hardly ever complain about itching (Strassburg, 2010). These results suggest that bilirubin is not a dominant pruritogen in cholestasis-associated pruritus.

Steroid Metabolites

Steroids like progesterone and estrogen might be involved in the pathogenesis of cholestasis-associated pruritus, particularly in the case of ICP. Progesterone and estrogen metabolites can be converted into steroids with a cholestatic potential (Reyes and Sjovall, 2000). In women with ICP, serum concentrations of progesterone metabolites (pregnanediol sulfates) are elevated at 35–41 weeks of gestation (Abu-Hayyeh et al., 2013; Meng et al., 1997). These progesterone metabolites have been implicated as potential pruritogens from analyses of urine samples from ICP cases (Abu-Hayyeh et al., 2016; Glantz et al., 2008). Progesterone metabolites can interact with the bile salt receptor FXR (Abu-Hayyeh et al., 2016; Abu-Hayyeh et al., 2013), the bile salt transporter NTCP (Abu-Hayyeh et al., 2010) and the bile salt export pump (BSEP) (Vallejo et al., 2006), thereby affecting bile salt homeostasis pathways and inhibiting hepatic bile salt uptake and efflux. This could possibly result in, or contribute to cholestasis and hypercholanemia.

Three pregnanediol sulfates are found at increased concentrations in plasma during gestational weeks 9–15, prior to symptom onset of ICP. These concentrations were (weakly) associated with itch severity and were able to differentiate between women with ICP compared to those with benign pruritus. One of these progesterone sulfates, 5 β -pregnan-3 α ,-20 α -diol-3-sulfate (PM3S), evoked a TGR5-dependent scratch response in mice (Abu-Hayyeh et al., 2016). Together, these results suggest that steroid metabolites might influence pruriception in cholestasis. Further research into steroid metabolites is needed to evaluate their mechanism in cholestasis-associated pruritus.

PAR2-Agonists

Protease-activated receptors (PARs) are implicated in somatosensory functions like itch and pain (Akiyama et al., 2015). They have been studied in relation to chronic itch in atopic dermatitis patients. PARs consist of four members, where PAR2 is highly expressed in keratinocytes and is thought to be involved in pruritus caused by atopic dermatitis (Akiyama et al., 2015; Briot et al., 2009; Kempkes et al., 2014; Steinhoff et al., 2003). Activation of PAR2 in keratinocytes induces the release of pruritogenic cytokines that can induce itch through channels on sensory neurons (Akiyama et al., 2015). A recent study of Zhao et al. suggested that PAR2 mediates itch via TRPV3 signaling. Both PAR2 and TRPV1 were shown to be upregulated in the skin of patients with atopic dermatitis and in mouse models for atopic dermatitis (Zhao et al., 2020).

PAR2 can be activated by the agonist SLIGRL and the proteases trypsin and tryptase. Skin application of those compounds have been shown to induce itch and scratch behavior in humans and in mice, respectively (Costa et al., 2008; Shimada et al., 2006; Steinhoff et al., 2003; Thomsen et al., 2002; Ui et al., 2006). However, the shorter peptide, SLIGR, which also activates PAR2, did not induce scratching behavior but rather induced thermal hyperalgesia (Liu et al., 2011). Tryptase is a mast cell derived protease, which has been evaluated in cholestasis-associated pruritus, but a correlation with pruritus intensity could not be found (Kremer et al., 2010). However, other proteases might be involved in cholestasis-associated pruritus that could signal through PAR2 and illicit itch sensation.

Gut Microbiome

The first link between gut microbiota and liver pathogenesis was made by Pereira et al., who hypothesized that neutrophil activators involved in inflammatory bowel disease, pass from the inflamed colon to the liver via the enterohepatic circulation (Pereira et al., 1998). Biliary lactoferrin concentrations were increased in active ulcerative colitis and Crohn's disease, and fell with colectomy and disease remission. Patients with PSC are often also identified with inflammatory bowel disease. This suggests a potential role for gut-derived factors.

In a review of Li et al., several studies are discussed that suggest the important role of intestinal microbiota in the etiopathogenesis of cholestatic liver diseases by regulating metabolism and immune responses (Li et al., 2017). A study in 2016 showed a reduction of

several potential beneficial microbiota and enrichment of opportunistic pathogens in early-stage PBC patients compared to healthy controls (Li et al., 2017; Lv et al., 2016). Species richness was reduced in PBC patients, resulting in a shift in overall microbial diversity (Li et al., 2017; Tang et al., 2018). These results were ameliorated after ursodeoxycholic acid (UDCA) treatment. However, in both of these studies, cholestasis-associated pruritus was not measured. Probiotic studies have shown antipruritic effects in patients with atopic dermatitis (Matsumoto et al., 2007; Matsumoto et al., 2014).

Recently, Hegade et al. performed a study in which they compared PBC-patients suffering from pruritus with non-symptomatic PBC patients and healthy volunteers (Hegade et al., 2019). The gut microbiota showed no significant difference between those groups, suggesting that cholestasis-associated pruritus is not associated with a specific gut bacterial composition. However, after treatment with the ASBT-inhibitor linerixibat (GSK2330672), which reduces pruritus scores in PBC patients, fecal bacterial composition significantly changed from baseline. These changes might be due to the increased bile salt load in the colon resulting from ASBT inhibition (Hegade et al., 2017; Hegade et al., 2019). Together, these studies do not support a major role for gut microbiota in the pathology of cholestasis-associated pruritus.

Treatment of Cholestasis-Associated Pruritus

Evidence-Based Treatments

Evidence-based and experimental treatments for cholestasis-associated pruritus have recently been reviewed (Patel et al., 2019). Here, we will provide a brief overview. In general, treatment of pruritus can be associated with a considerable placebo effect when using subjective patient-reported outcomes (Darragh et al., 2015; Mayo et al., 2019). In line with European Association for the Study of the Liver (EASL, 2009), patients with PBC, but also various other cholestatic liver diseases, are treated with ursodeoxycholic acid (UDCA). UDCA exerts potent anticholestatic effects and improves biochemical surrogate markers of prognosis such as serum bilirubin or alkaline phosphatase (ALP), histological features (Beuers, 2006; Corpechot et al., 2000; Pares et al., 2000) and liver transplantation-free survival in PBC and, according to most recently reported preliminary data, also in PSC. A beneficial effect of UDCA on pruritus, however, was not reported in large trials.

The non-specific peroxisome proliferator-activated receptor (PPAR) agonist bezafibrate has most recently been shown to effectively improve severe to moderate pruritus in 74 patients with PSC and PBC in a prospective, randomized, double-blind, placebo-controlled trial (Bolier et al., 2017; de Vries et al., 2020). Bezafibrate has first been introduced as 2nd line treatment of PBC in Japan (Nakai et al., 2000) in combination with UDCA in patients incompletely responding to UDCA alone. Its anticholestatic effect and that of the PPAR α agonist fenofibrate has since then been documented in various small trials (Ghonem and Boyer, 2013), and finally in a prospective, randomized, placebo-controlled study in 100 patients with PBC treated over 2 years (Corpechot et al., 2018; Kremer et al., 2019; Verkade

et al., 2020) convincingly confirmed. In this trial, a minor antipruritic effect of bezafibrate represented one of the favorable secondary observations in PBC patients with mild pruritus. A case series describing an antipruritic effect of bezafibrate was published in 2018 (Reig et al., 2018). It remains unclear at present whether bezafibrate is superior to the selective PPAR α agonist fenofibrate, which also exerts anticholestatic and antipruritic effects in PBC. With its antipruritic, anticholestatic, and anti-inflammatory effects in fibrosing cholangiopathies such as PSC or PBC and its safety profile, bezafibrate—and potentially other PPAR agonists should become the 1st line treatment in cholestasis-associated pruritus in the future.

The antibiotic rifampicin is a potent pregnane X receptor (PXR) agonist, and, thereby, inducer of phase 1 and phase 2 biotransformation enzymes including cytochrome P4503A4, a major drug-metabolizing enzyme. Rifampicin is widely used to reduce cholestasis-associated pruritus and is recommended as a second line antipruritic agent (Khurana and Singh, 2006; Tandon et al., 2007). It induces biotransformation of many endogenous and exogenous compounds by phase 1 (e.g., hydroxylation) and phase 2 (e.g., glucuronidation) reactions (Acocella, 1983; Thebaut et al., 2018; Wietholtz et al., 1996) to more water-soluble molecules which can then be excreted via the renal route. Hypothetically, rifampicin could eliminate potential pruritogens from the (enterohepatic) circulation. The antibacterial effects of rifampicin on intestinal flora may alter metabolization and absorption of primary and secondary bile salts and pruritogens (Khurana and Singh, 2006). Additionally, rifampicin can downregulate *ATX* expression via PXR-dependent mechanisms and, thereby reduce formation of the pruritogen LPA (Kremer et al., 2012). Currently, it is still unclear whether one or the other of these effects plays a prominent role in the anti-pruritic action of rifampicin. Adverse effects of rifampicin include discoloring of body fluids and teeth and resistance against rifampicin-sensitive bacteria. Use of rifampicin for over more than 2 weeks may enhance the risk of hepatotoxicity and therefore requires strict guidance in patients with liver disease (EASL, 2009).

As a third-line treatment for pruritus, oral opioid antagonists like naltrexone and naloxone are used (Bergasa et al., 1995; Tandon et al., 2007; Terg et al., 2002). Endogenous opioids were found to be elevated in plasma of patients with acute liver disease and cirrhosis with ascites (Thornton and Losowsky, 1988) but Kremer et al. did not observe a correlation between itch and plasma opioid activity (Kremer et al., 2010). Opioid antagonists are able to reduce pruritus (Terg et al., 2002; Thornton and Losowsky, 1988); however, they also can cause severe side effects similar to opioid withdrawal symptoms (Mansour-Ghanaei et al., 2006) and should be started at low daily doses.

Patients that are resistant to above-mentioned treatments can receive the selective serotonin reuptake inhibitor (SSRI) sertraline as a fourth-line treatment (Mayo et al., 2007). This antidepressant can alter potential pruritic pathways involving serotonin.

In the past, cholestyramine was used as the first-line recommended treatment against pruritus. Cholestyramine is an anion-exchange resin that can bind and sequester numerous amphiphilic compounds including bile salts and other potential pruritogens in the intestine (Carey and Williams, 1961; Datta and Sherlock, 1966; Rust et al., 2000). Despite the long

history of use, evidence for efficacy of this treatment modality is moderate at best (EASL, 2009; European Association for the Study of the Liver. Electronic address and European Association for the Study of the, 2017). In addition, cholestyramine quite often causes gastrointestinal complaints. In a prospective, randomized, placebo-controlled trial, a more potent anion-exchange resin, colesevelam, was able to reduce plasma bile salt levels by nearly 50%, but no effect was observed on intensity of pruritus when compared to placebo (Kuiper et al., 2010). Therefore, we conclude that anion-exchange resins should not anymore be ranked among evidence-based, recommended treatment options and rather should have a role as escape medication when other (above discussed) antipruritic medications fail or are not tolerated.

Invasive Treatments

Invasive treatments are indicated for patients that do not respond to abovementioned medical drug therapies. Patients can undergo extracorporeal albumin dialysis (Pares et al., 2004; Pares et al., 2010), plasmapheresis (Alallam et al., 2008; Heerkens et al., 2019; Krawczyk et al., 2017; Pusch et al., 2006) and biliary drainage (Beuers et al., 2006; Hegade et al., 2016; Stapelbroek et al., 2006). These treatments, if effective, only last for days or a few weeks and in exceptional cases months, after which pruritus returns. Surgical partial internal/external biliary diversion can be a permanent solution in children when performed before advanced liver disease has developed (Verkade et al., 2020). Liver transplantation can be performed as a last resort for severe cholestasis-associated pruritus refractory to medical and invasive therapeutic approaches.

Experimental Treatments

Pruritogens might undergo enterohepatic circulation in a similar way as bile salts do. The reuptake of bile salts and other compounds in the ileum, through the intestinal apical sodium-dependent bile acid transporter (ASBT), can be reduced by the use of ASBT-inhibitors and can give a strong reduction of itch sensation in patients. Several inhibitory pharmaceuticals have been developed, which have minimal systemic absorption, thereby only affecting absorption in the intestine. More recent ASBT inhibitors studied include linerixibat (GSK2330672) (Hegade et al., 2017; Hegade et al., 2019), maralixibat (SHP625) (Mayo et al., 2019) and odevixibat (A4250) (Al-Dury et al., 2018). Clinical trials with cholestatic patients suffering from pruritus showed promising results in reducing pruritus, however not always significantly different from placebo treatment (Al-Dury et al., 2018; Hegade et al., 2017; Hegade et al., 2019; Mayo et al., 2019). Frequent adverse events were diarrhea and abdominal discomfort possibly due to increased bile salt load in the large intestine. Notably, the majority of these adverse events resolved over time in one of the studies (Mayo et al., 2019).

In a placebo-controlled trial the effect of the κ -opioid antagonist nalfurafine was found to mildly reduce cholestasis-associated itch in 318 patients with chronic cholestasis-associated itch without major side effects (Kumada et al., 2017).

Since serum ATX levels correlate well with itch intensity in cholestatic patients, and the ATX product LPA can act as a pruritogen (Kremer et al., 2016; Kremer et al., 2010), future therapy for cholestasis-associated pruritus might include ATX-inhibitors [reviewed in (Barbayianni et al., 2013)]. Current research on ATX and ATX-inhibition is predominantly performed in the cancer and inflammation fields (Castagna et al., 2016; Herr et al., 2020). The ATX-LPA axis is involved in multiple functions, including cell migration, angiogenesis and metastasis (Moolenaar and Perrakis, 2011). Future studies will have to study the safety and efficacy of ATX-inhibitors and their potential role in cholestasis-associated pruritus.

Challenges and future perspectives of cholestasis-associated pruritus research

Patients with cholestatic diseases often suffer from severe, chronic pruritus, which impairs their quality of life. It is up to researchers to unravel the mechanism(s) of cholestasis-associated pruritus, and even more importantly, to provide treatment options to reduce or resolve this burden.

This review highlights the fact that for cholestasis-associated pruritus there are many possible pruritogens, of which several are likely to play a role simultaneously. Due to different theories on the cause of cholestasis-associated pruritus, also different treatments exist of which none has yet shown to represent the final solution. Research in this field will have to continue in order to clarify the mechanism and to provide a satisfying treatment for patients with cholestasis-associated pruritus.

One of the biggest challenges in cholestasis-associated pruritus research is the possible multi-organ aspect. Besides the affected liver, the enterohepatic circulation plays a crucial role in cholestasis-associated pruritus. Multiple other organs play a potential role in cholestasis-associated pruritus, including the intestine which feeds the enterohepatic circulation with multiple (unknown) compounds, the blood with all its proteins, hormones and lipids, the skin with its receptors, barriers and nerves, and the brain of which still a lot is unknown. All of these aspects can affect the production, transport, accumulation, binding, metabolism, and excretion of potential pruritogen(s).

The next questions arise; are pruritogens produced in a higher amount in patients with cholestatic disease as compared to healthy people as a consequence of cholestasis, or are pruritogens present at all times and do they accumulate due to cholestatic circumstances? Is it possible that there is increased production of pruritogens as a secondary effect of accumulation of non-pruritogens? Bile formation is an important way of eliminating waste products; however, many compounds in bile are recirculated in the enterohepatic circulation, which opens the possibility of accumulation during cholestasis. We can interrupt the complete enterohepatic circulation (by anion-exchange resins, nasobiliary drainage, ASBT-inhibition or partial external biliary diversion (PEBD)), or remove hypothetical compounds from the circulation (by plasmapheresis or albumin dialysis), but we still do not know for which compounds elimination is essential. Based on the most recent studies, we

would expect higher levels of pruritogens in plasma (and possibly even in bile) of patients with chronic pruritus compared to people without chronic pruritus, since elimination or filtration provides relief of pruritus. However, the possibility remains that pruritus is not caused by a change in the level of pruritogens but rather the sensitivity to them. Thus, in patients with cholestasis-associated pruritus there might be a change in sensory signaling involving receptor expression in neurons or the skin. These alternatives need to be investigated in detail still.

Another challenge in pruritus research is the way of measuring pruritus (in mice and men), which is best analyzed in a mechanic or sensory way, but mental experience is also an important factor in human patients with pruritus and makes itch-perception a subjective measure. The amount or intensity of pruritus is preferably expressed in an objective value, which is comparable between individuals, and not affected by side effects like emotions, mental stress or fatigue. An important challenge in pruritus research is correcting for the placebo effect of possible treatment. Several studies have shown that treatment with placebo already improves itch sensation by a great deal. Hence, a placebo group should always be included.

Identification of pruritogens involved in cholestasis might find its future in untargeted metabolomic research. All metabolites in bile, serum and possibly skin can be measured and compared between groups. The question remains whether all metabolites can yet be identified and traced back to their origin, but future research will expand the pool of identifiable metabolites. The presence or concentration of compounds might overlap in different patient groups and in individual patients. This approach might clarify in which combination certain compounds are involved. Identification of compounds (possibly pruritogens) will open a way to unravel their involvement in the mechanisms of cholestasis-associated pruritus and develop more effective treatment options for those patients.

Outline of this thesis

In this thesis, we have explored several aspects of the mechanisms of cholestasis-associated pruritus (itch). Next to fatigue, pruritus is the most frequent symptom in patients with chronic cholestatic disorders and has serious impact on their lives. As the mechanism of cholestasis-associated pruritus is still largely unknown, it was our aim to shed some more light on this potentially debilitating clinical symptom.

In order to study cholestasis-associated pruritus, our aim was to mimic cholestasis in a mouse model, to measure their scratch activity and to influence the process in order to unravel the mechanisms. In **Chapter 2**, we measured scratch activity in cholestatic *Atp8b1* mutant mice, a model for Progressive Familial Intrahepatic Cholestasis type 1, and wild type mice (WT) with alpha-naphthylisothiocyanate (ANIT)-induced cholestasis. As we have previously shown, plasma autotaxin (ATX) activity correlates with the severity of pruritus in cholestatic patients. To further investigate this correlation, ATX was overexpressed in these mice by transduction with AAV-ATX and the effect on scratch activity was measured.

During cholestasis, multiple biliary compounds can accumulate in the systemic circulation, including bile salts, bile salt-like molecules and sulfated progesterone metabolites. These molecules have the possibility to affect ATX activity and expression and play an important role in causing pruritus. In **Chapter 3**, we measured ATX activity and expression under the influence of those molecules including the role of the ATX-product LPA.

We hypothesized that during cholestasis, pruritogens that are normally excreted into bile, accumulate in the enterohepatic circulation, from which they can be successfully removed by biliary diversion such as nasobiliary drainage. In **Chapter 4**, we screened diverted bile of patients with cholestasis-associated pruritus for specific compounds that are able to activate itch-associated channels.

Lysophosphatidic acid (LPA) is involved in pain and itch signaling, among other processes, whereby the LPA₅ receptor is suggested to play a prominent role in neuropathic and inflammatory pain as well as in itch signaling. In **Chapter 5**, we focused on the LPAR₅-antagonist compound 3 (cpd3) and its ability to affect pain and itch signaling, both *in vitro* and *in vivo*.

REFERENCES

- Abu-Hayyeh, S., P. Martinez-Becerra, S.H. Sheikh Abdul Kadir, C. Selden, M.R. Romero, M. Rees, H.U. Marschall, J.J. Marin, and C. Williamson. 2010. Inhibition of Na⁺-taurocholate Co-transporting polypeptide-mediated bile acid transport by cholestatic sulfated progesterone metabolites. *J Biol Chem.* 285:16504-16512.
- Abu-Hayyeh, S., C. Ovadia, T. Lieu, D.D. Jensen, J. Chambers, P.H. Dixon, A. Lovgren-Sandblom, R. Bolier, D. Tolenaars, A.E. Kremer, A. Syngelaki, M. Noori, D. Williams, J.J. Marin, M.J. Monte, K.H. Nicolaidis, U. Beuers, R. Oude-Elferink, P.T. Seed, L. Chappell, H.U. Marschall, N.W. Bunnett, and C. Williamson. 2016. Prognostic and mechanistic potential of progesterone sulfates in intrahepatic cholestasis of pregnancy and pruritus gravidarum. *Hepatology (Baltimore, Md.)*. 63:1287-1298.
- Abu-Hayyeh, S., G. Papacleovoulou, A. Lovgren-Sandblom, M. Tahir, O. Oduwole, N.A. Jamaludin, S. Ravat, V. Nikolova, J. Chambers, C. Selden, M. Rees, H.U. Marschall, M.G. Parker, and C. Williamson. 2013. Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites inhibit farnesoid X receptor resulting in a cholestatic phenotype. *Hepatology (Baltimore, Md.)*. 57:716-726.
- Acocella, G. 1983. Pharmacokinetics and metabolism of rifampin in humans. *Rev Infect Dis.* 5 Suppl 3:S428-432.
- Akiyama, T., M.I. Carstens, and E. Carstens. 2010. Facial injections of pruritogens and algogens excite partly overlapping populations of primary and second-order trigeminal neurons in mice. *J Neurophysiol.* 104:2442-2450.
- Akiyama, T., M. Ivanov, M. Nagamine, A. Davoodi, M.I. Carstens, A. Ikoma, F. Cevikbas, C. Kempkes, J. Buddenkotte, M. Steinhoff, and E. Carstens. 2016. Involvement of TRPV4 in Serotonin-Evoked Scratching. *The Journal of investigative dermatology.* 136:154-160.
- Akiyama, T., E.A. Lerner, and E. Carstens. 2015. Protease-activated receptors and itch. *Handb Exp Pharmacol.* 226:219-235.
- Al-Dury, S., A. Wahlstrom, S. Wahlin, J. Langedijk, R.O. Elferink, M. Stahlman, and H.U. Marschall. 2018. Pilot study with IBAT inhibitor A4250 for the treatment of cholestatic pruritus in primary biliary cholangitis. *Scientific reports.* 8:6658.
- Alallam, A., D. Barth, and E.J. Heathcote. 2008. Role of plasmapheresis in the treatment of severe pruritus in pregnant patients with primary biliary cirrhosis: case reports. *Can J Gastroenterol.* 22:505-507.
- Alemi, F., E. Kwon, D.P. Poole, T. Lieu, V. Lyo, F. Cattaruzza, F. Cevikbas, M. Steinhoff, R. Nassini, S. Materazzi, R. Guerrero-Alba, E. Valdez-Morales, G.S. Cottrell, K. Schoonjans, P. Geppetti, S.J. Vanner, N.W. Bunnett, and C.U. Corvera. 2013. The TGR5 receptor mediates bile acid-induced itch and analgesia. *J Clin Invest.* 123:1513-1530.
- Amanesi, S., G.S. Cottrell, L. Divino, K. Chapman, E.F. Grady, F. Bautista, R. Karanjia, C. Barajas-Lopez, S. Vanner, N. Vergnolle, and N.W. Bunnett. 2006. Protease-activated receptor 2 sensitizes TRPV1 by protein kinase Cepsilon- and A-dependent mechanisms in rats and mice. *J Physiol.* 575:555-571.
- Andersen, H.H., J. Elberling, H. Solvsten, G. Yosipovitch, and L. Arendt-Nielsen. 2017. Nonhistaminergic and mechanical itch sensitization in atopic dermatitis. *Pain.* 158:1780-1791.
- Andrew, D., and A.D. Craig. 2001. Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. *Nat Neurosci.* 4:72-77.
- Arrese, M., R.I. Macias, O. Briz, M.J. Perez, and J.J. Marin. 2008. Molecular pathogenesis of intrahepatic cholestasis of pregnancy. *Expert Rev Mol Med.* 10:e9.
- Atanassoff, P.G., S.J. Brull, J. Zhang, K. Greenquist, D.G. Silverman, and R.H. Lamotte. 1999. Enhancement of experimental pruritus and mechanically evoked dysesthesiae with local anesthesia. *Somatosensory & motor research.* 16:291-298.

- Ballantyne, J.C., A.B. Loach, and D.B. Carr. 1988. Itching after epidural and spinal opiates. *Pain*. 33:149-160.
- Barbayanni, E., V. Magrioti, P. Moutevelis-Minakakis, and G. Kokotos. 2013. Autotaxin inhibitors: a patent review. *Expert Opin Ther Pat*. 23:1123-1132.
- Bartholomew, T.C., J.A. Summerfield, B.H. Billing, A.M. Lawson, and K.D. Setchell. 1982. Bile acid profiles of human serum and skin interstitial fluid and their relationship to pruritus studied by gas chromatography-mass spectrometry. *Clin Sci (Lond)*. 63:65-73.
- Beck, P.W., and H.O. Handwerker. 1974. Bradykinin and serotonin effects on various types of cutaneous nerve fibers. *Pflugers Arch*. 347:209-222.
- Bell, J.K., D.S. McQueen, and J.L. Rees. 2004. Involvement of histamine H4 and H1 receptors in scratching induced by histamine receptor agonists in Balb C mice. *Br J Pharmacol*. 142:374-380.
- Bergasa, N.V. 2018. The pruritus of cholestasis: From bile acids to opiate agonists: Relevant after all these years. *Med Hypotheses*. 110:86-89.
- Bergasa, N.V., S.L. Sabol, W.S. Young, 3rd, D.E. Kleiner, and E.A. Jones. 1995. Cholestasis is associated with preproenkephalin mRNA expression in the adult rat liver. *Am J Physiol*. 268:G346-354.
- Bergasa, N.V., T.L. Talbot, D.W. Alling, J.M. Schmitt, E.C. Walker, B.L. Baker, J.C. Korenman, Y. Park, J.H. Hoofnagle, and E.A. Jones. 1992. A controlled trial of naloxone infusions for the pruritus of chronic cholestasis. *Gastroenterology*. 102:544-549.
- Bernstein, J.E., R.M. Swift, K. Soltani, and A.L. Lorincz. 1982. Antipruritic effect of an opiate antagonist, naloxone hydrochloride. *The Journal of investigative dermatology*. 78:82-83.
- Beuers, U. 2006. Drug insight: Mechanisms and sites of action of ursodeoxycholic acid in cholestasis. *Nat Clin Pract Gastroenterol Hepatol*. 3:318-328.
- Beuers, U., G. Gerken, and T. Puhl. 2006. Biliary drainage transiently relieves intractable pruritus in primary biliary cirrhosis. *Hepatology (Baltimore, Md)*. 44:280-281.
- Beuers, U., A.E. Kremer, R. Bolier, and R.P.J.O. Elferink. 2014. Pruritus in cholestasis: Facts and fiction. *Hepatology*. 60:399-407.
- Beuers, U., U. Spengler, F.M. Zwiebel, J. Pauletzki, S. Fischer, and G. Paumgartner. 1992. Effect of ursodeoxycholic acid on the kinetics of the major hydrophobic bile acids in health and in chronic cholestatic liver disease. *Hepatology (Baltimore, Md)*. 15:603-608.
- Bockaert, J., S. Claeysen, C. Becamel, A. Dumuis, and P. Marin. 2006. Neuronal 5-HT metabotropic receptors: fine-tuning of their structure, signaling, and roles in synaptic modulation. *Cell Tissue Res*. 326:553-572.
- Bolier, R., E.S. de Vries, A. Pares, J. Helder, E.M. Kemper, K. Zwinderman, R.P.O. Elferink, U. Beuers, and G. Netherlands Association for the Study of the Liver Cholestatic Liver Diseases Study. 2017. Fibrates for the treatment of cholestatic itch (FITCH): study protocol for a randomized controlled trial. *Trials*. 18:230.
- Bolier, R., D. Tolenaars, A.E. Kremer, J. Saris, A. Pares, J. Verheij, P.J. Bosma, U. Beuers, and R.P.J. Oude Elferink. 2016. Enteroendocrine cells are a potential source of serum autotaxin in men. *Biochimica et biophysica acta*. 1862:696-704.
- Briot, A., C. Deraison, M. Lacroix, C. Bonnart, A. Robin, C. Besson, P. Dubus, and A. Hovnanian. 2009. Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J Exp Med*. 206:1135-1147.
- Browning, J., B. Combes, and M.J. Mayo. 2003. Long-term efficacy of sertraline as a treatment for cholestatic pruritus in patients with primary biliary cirrhosis. *Am J Gastroenterol*. 98:2736-2741.
- Carey, J.B., Jr. 1961. Bile acids in the serum of jaundiced patients. *Gastroenterology*. 41:285-287.

- Carey, J.B., Jr., and G. Williams. 1961. Relief of the pruritus of jaundice with a bile-acid sequestering resin. *JAMA*. 176:432-435.
- Castagna, D., D.C. Budd, S.J. Macdonald, C. Jamieson, and A.J. Watson. 2016. Development of Autotaxin Inhibitors: An Overview of the Patent and Primary Literature. *J Med Chem*. 59:5604-5621.
- Choi, J.W., and J. Chun. 2013. Lysophospholipids and their receptors in the central nervous system. *Biochimica et biophysica acta*. 1831:20-32.
- Cipriani, S., B. Renga, C. D'Amore, M. Simonetti, A.A. De Tursi, A. Carino, M.C. Monti, V. Sepe, A. Zampella, and S. Fiorucci. 2015. Impaired Itching Perception in Murine Models of Cholestasis Is Supported by Dysregulation of GPBAR1 Signaling. *PLoS One*. 10:e0129866.
- Corpechot, C., F. Carrat, A.M. Bonnard, R.E. Poupon, and R. Poupon. 2000. The effect of ursodeoxycholic acid therapy on liver fibrosis progression in primary biliary cirrhosis. *Hepatology (Baltimore, Md.)*. 32:1196-1199.
- Corpechot, C., O. Chazouilleres, A. Rousseau, A. Le Gruyer, F. Habersetzer, P. Mathurin, O. Gorla, P. Potier, A. Minello, C. Silvain, A. Abergel, M. Debette-Gratien, D. Larrey, O. Roux, J.P. Bronowicki, J. Boursier, V. de Ledinghen, A. Heurgue-Berlot, E. Nguyen-Khac, F. Zoulim, I. Ollivier-Hourmand, J.P. Zarski, G. Nkontchou, S. Lemoine, L. Humbert, D. Rainteau, G. Lefevre, L. de Chaisemartin, S. Chollet-Martin, F. Gaouar, F.H. Admane, T. Simon, and R. Poupon. 2018. A Placebo-Controlled Trial of Bezafibrate in Primary Biliary Cholangitis. *N Engl J Med*. 378:2171-2181.
- Costa, R., D.M. Marotta, M.N. Manjavachi, E.S. Fernandes, J.F. Lima-Garcia, A.F. Paszcuk, N.L. Quintao, L. Juliano, S.D. Brain, and J.B. Calixto. 2008. Evidence for the role of neurogenic inflammation components in trypsin-elicited scratching behaviour in mice. *Br J Pharmacol*. 154:1094-1103.
- Cousins, M.J., and L.E. Mather. 1984. Intrathecal and epidural administration of opioids. *Anesthesiology*. 61:276-310.
- Cowan, A., G.B. Kehner, and S. Inan. 2015. Targeting Itch with Ligands Selective for κ Opioid Receptors. *In Pharmacology of Itch*. A. Cowan and G. Yosipovitch, editors. Springer Berlin Heidelberg, Berlin, Heidelberg. 291-314.
- Darragh, M., J.W. Chang, R.J. Booth, and N.S. Considine. 2015. The placebo effect in inflammatory skin reactions: the influence of verbal suggestion on itch and weal size. *J Psychosom Res*. 78:489-494.
- Datta, D.V., and S. Sherlock. 1966. Cholestyramine for long term relief of the pruritus complicating intrahepatic cholestasis. *Gastroenterology*. 50:323-332.
- Davidson, S., X. Zhang, C.H. Yoon, S.G. Khasabov, D.A. Simone, and G.J. Giesler, Jr. 2007. The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. *J Neurosci*. 27:10007-10014.
- De Logu, F., R. Maglie, M. Titiz, G. Poli, L. Landini, M. Marini, D. Souza Monteiro de Araujo, G. De Siena, M. Montini, D.A. Cabrini, M.F. Otuki, P.L. Pawloski, E. Antiga, T. Tuccinardi, J.B. Calixto, P. Geppetti, R. Nassini, and E. André. 2023. miRNA-203b-3p Induces Acute and Chronic Pruritus through 5-HTR2B and TRPV4. *Journal of Investigative Dermatology*. 143:142-153.e110.
- de Vries, E., R. Bolier, J. Goet, A. Pares, J. Verbeek, M. de Vree, J. Drenth, K. van Erpecum, K. van Nieuwkerk, F. van der Heide, N. Mostafavi, J. Helder, C. Ponsioen, R. Oude Elferink, H. van Buuren, U. Beuers, and G. Netherlands Association for the Study of the Liver-Cholestasis Working. 2020. Fibrates for Itch (FITCH) in Fibrosing Cholangiopathies: A Double-Blind, Randomized, Placebo-Controlled Trial. *Gastroenterology*.
- Dong, X., and X. Dong. 2018. Peripheral and Central Mechanisms of Itch. *Neuron*. 98:482-494.
- Dong, X., S. Han, M.J. Zylka, M.I. Simon, and D.J. Anderson. 2001. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell*. 106:619-632.
- Dziki, A.J., S. Batzri, J.W. Harmon, and M. Molloy. 1995. Cellular hypercalcemia is an early event in deoxycholate injury of rabbit gastric mucosal cells. *Am J Physiol*. 269:G287-296.

- EASL. 2009. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *Journal of hepatology*. 51:237-267.
- Erlinger, S. 2015. NTCP deficiency: a new inherited disease of bile acid transport. *Clin Res Hepatol Gastroenterol*. 39:7-8.
- European Association for the Study of the Liver. Electronic address, e.e.e., and L. European Association for the Study of the. 2017. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. *Journal of hepatology*. 67:145-172.
- Flegel, C., N. Schobel, J. Altmuller, C. Becker, A. Tannapfel, H. Hatt, and G. Gisselmann. 2015. RNA-Seq Analysis of Human Trigeminal and Dorsal Root Ganglia with a Focus on Chemoreceptors. *PLoS One*. 10:e0128951.
- Freedman, M.R., R.T. Holzbach, and D.R. Ferguson. 1981. Pruritus in cholestasis: no direct causative role for bile acid retention. *The American journal of medicine*. 70:1011-1016.
- Fujimori, N., T. Umemura, T. Kimura, N. Tanaka, A. Sugiura, T. Yamazaki, S. Joshita, M. Komatsu, Y. Usami, K. Sano, K. Igarashi, A. Matsumoto, and E. Tanaka. 2018. Serum autotaxin levels are correlated with hepatic fibrosis and ballooning in patients with non-alcoholic fatty liver disease. *World journal of gastroenterology*. 24:1239-1249.
- Fujino, H., M. Tanaka, M. Imamura, K. Morio, A. Ono, T. Nakahara, E. Murakami, T. Kawaoka, S. Takahashi, D. Miki, M. Tsuge, A. Hiramatsu, H. Aikata, C.N. Hayes, and K. Chayama. 2019. Pruritus in patients with chronic liver disease and serum autotaxin levels in patients with primary biliary cholangitis. *BMC Gastroenterol*. 19:169.
- Geenes, V., and C. Williamson. 2009. Intrahepatic cholestasis of pregnancy. *World journal of gastroenterology*. 15:2049-2066.
- Ghent, C.N., J.R. Bloomer, and G. Klatskin. 1977. Elevations in Skin Tissue Levels of Bile-Acids in Human Cholestasis - Relation to Serum Levels and to Pruritus. *Gastroenterology*. 73:1125-1130.
- Ghonem, N.S., and J.L. Boyer. 2013. Fibrates as adjuvant therapy for chronic cholestatic liver disease: its time has come. *Hepatology (Baltimore, Md.)*. 57:1691-1693.
- Gittlen, S.D., E.S. Schulman, and W.C. Maddrey. 1990. Raised histamine concentrations in chronic cholestatic liver disease. *Gut*. 31:96-99.
- Glantz, A., S.J. Reilly, L. Benthin, F. Lammert, L.A. Mattsson, and H.U. Marschall. 2008. Intrahepatic cholestasis of pregnancy: Amelioration of pruritus by UDCA is associated with decreased progesterone disulphates in urine. *Hepatology (Baltimore, Md.)*. 47:544-551.
- Golbar, H.M., T. Izawa, C. Ichikawa, M. Tanaka, V. Juniantito, O. Sawamoto, M. Kuwamura, and J. Yamate. 2013. Slowly progressive cholangiofibrosis induced in rats by alpha-naphthylisothiocyanate (ANIT), with particular references to characteristics of macrophages and myofibroblasts. *Exp Toxicol Pathol*. 65:825-835.
- Gupta, M.A., and A.K. Gupta. 1999. Depression modulates pruritus perception. A study of pruritus in psoriasis, atopic dermatitis and chronic idiopathic urticaria. *Ann N Y Acad Sci*. 885:394-395.
- Hashimoto, T., H. Ohata, and K. Momose. 2004. Itch-scratch responses induced by lysophosphatidic acid in mice. *Pharmacology*. 72:51-56.
- Hausmann, J., S. Kamtekar, E. Christodoulou, J.E. Day, T. Wu, Z. Fulkerson, H.M. Albers, L.A. van Meeteren, A.J. Houben, L. van Zeijl, S. Jansen, M. Andries, T. Hall, L.E. Pegg, T.E. Benson, M. Kasiem, K. Harlos, C.W. Kooi, S.S. Smyth, H. Ovaa, M. Bollen, A.J. Morris, W.H. Moolenaar, and A. Perrakis. 2011. Structural basis of substrate discrimination and integrin binding by autotaxin. *Nat Struct Mol Biol*. 18:198-204.
- Heerkens, M., S. Dedden, H. Scheepers, P. Van Paassen, A. Masclee, C. de Die-Smulders, S.W.M. Olde Damink, F.G. Schaap, P. Jansen, G. Koek, U. Beuers, and J. Verbeek. 2019. Effect of Plasmapheresis on Cholestatic Pruritus and Autotaxin Activity During Pregnancy. *Hepatology (Baltimore, Md.)*. 69:2707-2710.

- Hegade, V.S., S.F. Kendrick, R.L. Dobbins, S.R. Miller, D. Thompson, D. Richards, J. Storey, G.E. Dukes, M. Corrigan, R.P. Oude Elferink, U. Beuers, G.M. Hirschfield, and D.E. Jones. 2017. Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study. *Lancet*. 389:1114-1123.
- Hegade, V.S., M. Krawczyk, A.E. Kremer, J. Kuczka, F. Gaouar, E.M. Kuiper, H.R. van Buuren, F. Lammert, C. Corpechot, and D.E. Jones. 2016. The safety and efficacy of nasobiliary drainage in the treatment of refractory cholestatic pruritus: a multicentre European study. *Aliment Pharmacol Ther*. 43:294-302.
- Hegade, V.S., A. Pechlivanis, J.A.K. McDonald, D. Rees, M. Corrigan, G.M. Hirschfield, S.D. Taylor-Robinson, E. Holmes, J.R. Marchesi, S. Kendrick, and D.E. Jones. 2019. Autotaxin, bile acid profile and effect of ileal bile acid transporter inhibition in primary biliary cholangitis patients with pruritus. *Liver Int*. 39:967-975.
- Herr, D.R., W.S. Chew, R.L. Satish, and W.Y. Ong. 2020. Pleotropic Roles of Autotaxin in the Nervous System Present Opportunities for the Development of Novel Therapeutics for Neurological Diseases. *Mol Neurobiol*. 57:372-392.
- Hoyer, D., and G. Martin. 1997. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. *Neuropharmacology*. 36:419-428.
- Ikoma, A., H. Handwerker, Y. Miyachi, and M. Schmelz. 2005. Electrically evoked itch in humans. *Pain*. 113:148-154.
- Ikoma, A., M. Steinhoff, S. Stander, G. Yosipovitch, and M. Schmelz. 2006. The neurobiology of itch. *Nat Rev Neurosci*. 7:535-547.
- Imamachi, N., G.H. Park, H. Lee, D.J. Anderson, M.I. Simon, A.I. Basbaum, and S.K. Han. 2009. TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*. 106:11330-11335.
- Jankowski, M. 2011. Autotaxin: its role in biology of melanoma cells and as a pharmacological target. *Enzyme Res*. 2011:194857.
- Johanek, L.M., R.A. Meyer, R.M. Friedman, K.W. Greenquist, B. Shim, J. Borzan, T. Hartke, R.H. LaMotte, and M. Ringkamp. 2008. A role for polymodal C-fiber afferents in nonhistaminergic itch. *J Neurosci*. 28:7659-7669.
- Jones, E.A., and N.V. Bergasa. 2000. Evolving concepts of the pathogenesis and treatment of the pruritus of cholestasis. *Can J Gastroenterol*. 14:33-40.
- Jones, E.A., and L.R. Dekker. 2000. Florid opioid withdrawal-like reaction precipitated by naltrexone in a patient with chronic cholestasis. *Gastroenterology*. 118:431-432.
- Joshi, N., J.L. Ray, A.K. Kopec, and J.P. Luyendyk. 2017. Dose-dependent effects of alpha-naphthylisothiocyanate disconnect biliary fibrosis from hepatocellular necrosis. *J Biochem Mol Toxicol*. 31:1-7.
- Joshita, S., Y. Ichikawa, T. Umemura, Y. Usami, A. Sugiura, S. Shibata, T. Yamazaki, N. Fujimori, M. Komatsu, A. Matsumoto, K. Igarashi, M. Ota, and E. Tanaka. 2018a. Serum autotaxin is a useful liver fibrosis marker in patients with chronic hepatitis B virus infection. *Hepatology research : the official journal of the Japan Society of Hepatology*. 48:275-285.
- Joshita, S., T. Umemura, Y. Usami, Y. Yamashita, G.L. Norman, A. Sugiura, T. Yamazaki, N. Fujimori, T. Kimura, A. Matsumoto, K. Igarashi, K. Yoshizawa, M. Ota, and E. Tanaka. 2018b. Serum Autotaxin Is a Useful Disease Progression Marker in Patients with Primary Biliary Cholangitis. *Scientific reports*. 8:8159.
- Jurcakova, D., F. Ru, M. Kollarik, H. Sun, J. Krajewski, and B.J. Udem. 2018. Voltage-Gated Sodium Channels Regulating Action Potential Generation in Itch-, Nociceptive-, and Low-Threshold Mechanosensitive Cutaneous C-Fibers. *Mol Pharmacol*. 94:1047-1056.

- Kamimura, K., T. Yokoo, H. Kamimura, A. Sakamaki, S. Abe, A. Tsuchiya, M. Takamura, H. Kawai, S. Yamagiwa, and S. Terai. 2017. Long-term efficacy and safety of nalfurafine hydrochloride on pruritus in chronic liver disease patients: Patient-reported outcome based analyses. *PLoS One*. 12:e0178991.
- Kempkes, C., J. Buddenkotte, F. Cevikbas, T. Buhl, and M. Steinhoff. 2014. Role of PAR-2 in Neuroimmune Communication and Itch. *In Itch: Mechanisms and Treatment*. E. Carstens and T. Akiyama, editors, Boca Raton (FL).
- Keune, W.J., J. Hausmann, R. Bolier, D. Tolenaars, A. Kremer, T. Heidebrecht, R.P. Joosten, M. Sunkara, A.J. Morris, E. Matas-Rico, W.H. Moolenaar, R.P. Oude Elferink, and A. Perrakis. 2016. Steroid binding to Autotaxin links bile salts and lysophosphatidic acid signalling. *Nat Commun*. 7:11248.
- Khurana, S., and P. Singh. 2006. Rifampin is safe for treatment of pruritus due to chronic cholestasis: a meta-analysis of prospective randomized-controlled trials. *Liver Int*. 26:943-948.
- Kittaka, H., and M. Tominaga. 2017. The molecular and cellular mechanisms of itch and the involvement of TRP channels in the peripheral sensory nervous system and skin. *Allergol Int*. 66:22-30.
- Kittaka, H., K. Uchida, N. Fukuta, and M. Tominaga. 2017. Lysophosphatidic acid-induced itch is mediated by signalling of LPA5 receptor, phospholipase D and TRPA1/TRPV1. *J Physiol*. 595:2681-2698.
- Klein, A., M.I. Carstens, and E. Carstens. 2011. Facial injections of pruritogens or algogens elicit distinct behavior responses in rats and excite overlapping populations of primary sensory and trigeminal subnucleus caudalis neurons. *J Neurophysiol*. 106:1078-1088.
- Koga, K., T. Chen, X.Y. Li, G. Descalzi, J. Ling, J. Gu, and M. Zhuo. 2011. Glutamate acts as a neurotransmitter for gastrin releasing peptide-sensitive and insensitive itch-related synaptic transmission in mammalian spinal cord. *Mol Pain*. 7:47.
- Krawczyk, M., R. Liebe, M. Wasilewicz, E. Wunsch, J. Raszeja-Wyszomirska, and P. Milkiewicz. 2017. Plasmapheresis exerts a long-lasting antipruritic effect in severe cholestatic itch. *Liver Int*. 37:743-747.
- Kremer, A.E., U. Beuers, R.P. Oude-Elferink, and T. Puhl. 2008. Pathogenesis and treatment of pruritus in cholestasis. *Drugs*. 68:2163-2182.
- Kremer, A.E., E. Gonzales, F.G. Schaap, R.P. Oude Elferink, E. Jacquemin, and U. Beuers. 2016. Serum Autotaxin Activity Correlates With Pruritus in Pediatric Cholestatic Disorders. *J Pediatr Gastroenterol Nutr*. 62:530-535.
- Kremer, A.E., A. Le Cleac'h, S. Lemoine, K. Wolf, L. De Chaisemartin, S. Chollet-Martin, L. Humbert, D. Rainteau, R. Poupon, A. Rousseau, O. Chazouilleres, and C. Corpechot. 2019. Antipruritic effect of bezafibrate and serum autotaxin measures in patients with primary biliary cholangitis. *Gut*. 68:1902-1903.
- Kremer, A.E., J.J. Martens, W. Kulik, F. Rueff, E.M. Kuiper, H.R. van Buuren, K.J. van Erpecum, J. Kondrackiene, J. Prieto, C. Rust, V.L. Geenes, C. Williamson, W.H. Moolenaar, U. Beuers, and R.P. Oude Elferink. 2010. Lysophosphatidic acid is a potential mediator of cholestatic pruritus. *Gastroenterology*. 139:1008-1018, 1018 e1001.
- Kremer, A.E., R. van Dijk, P. Leckie, F.G. Schaap, E.M. Kuiper, T. Mettang, K.S. Reiners, U. Raap, H.R. van Buuren, K.J. van Erpecum, N.A. Davies, C. Rust, A. Engert, R. Jalan, R.P. Oude Elferink, and U. Beuers. 2012. Serum autotaxin is increased in pruritus of cholestasis, but not of other origin, and responds to therapeutic interventions. *Hepatology (Baltimore, Md.)*. 56:1391-1400.
- Kuiper, E.M., K.J. van Erpecum, U. Beuers, B.E. Hansen, H.B. Thio, R.A. de Man, H.L. Janssen, and H.R. van Buuren. 2010. The potent bile acid sequestrant colesvelam is not effective in cholestatic pruritus: results of a double-blind, randomized, placebo-controlled trial. *Hepatology (Baltimore, Md.)*. 52:1334-1340.
- Kumada, H., H. Miyakawa, T. Muramatsu, N. Ando, T. Oh, K. Takamori, and H. Nakamoto. 2017. Efficacy of nalfurafine hydrochloride in patients with chronic liver disease with refractory pruritus:

- A randomized, double-blind trial. *Hepatology research : the official journal of the Japan Society of Hepatology*. 47:972-982.
- Kumagai, H., T. Ebata, K. Takamori, T. Muramatsu, H. Nakamoto, and H. Suzuki. 2010. Effect of a novel kappa-receptor agonist, nalfurafine hydrochloride, on severe itch in 337 haemodialysis patients: a Phase III, randomized, double-blind, placebo-controlled study. *Nephrol Dial Transplant*. 25:1251-1257.
- Kushnir-Sukhov, N.M., J.M. Brown, Y. Wu, A. Kirshenbaum, and D.D. Metcalfe. 2007. Human mast cells are capable of serotonin synthesis and release. *J Allergy Clin Immunol*. 119:498-499.
- Lagerstrom, M.C., K. Rogoz, B. Abrahamsen, E. Persson, B. Reinius, K. Nordenankar, C. Olund, C. Smith, J.A. Mendez, Z.F. Chen, J.N. Wood, A. Wallen-Mackenzie, and K. Kullander. 2010. VGLUT2-dependent sensory neurons in the TRPV1 population regulate pain and itch. *Neuron*. 68:529-542.
- Langedijk, J., R. Bolier, D. Tolenaars, L. Ten Bloemendaal, S. Duijst, D. de Waart, U. Beuers, P. Bosma, and R.O. Elferink. 2021. Reduced spontaneous itch in mouse models of cholestasis. *Scientific reports*. 11:6127.
- Lau, B.W., M. Colella, W.C. Ruder, M. Ranieri, S. Curci, and A.M. Hofer. 2005. Deoxycholic acid activates protein kinase C and phospholipase C via increased Ca²⁺ entry at plasma membrane. *Gastroenterology*. 128:695-707.
- Li, Y., R. Tang, P.S.C. Leung, M.E. Gershwin, and X. Ma. 2017. Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases. *Autoimmun Rev*. 16:885-896.
- Lieu, T., G. Jayaweera, P. Zhao, D.P. Poole, D. Jensen, M. Grace, P. McIntyre, R. Bron, Y.M. Wilson, M. Krappitz, S. Haerteis, C. Korbmacher, M.S. Steinhoff, R. Nassini, S. Materazzi, P. Geppetti, C.U. Corvera, and N.W. Bunnett. 2014. The bile acid receptor TGR5 activates the TRPA1 channel to induce itch in mice. *Gastroenterology*. 147:1417-1428.
- Liu, Q., Z. Tang, L. Surdenikova, S. Kim, K.N. Patel, A. Kim, F. Ru, Y. Guan, H.J. Weng, Y. Geng, B.J. Undem, M. Kollarik, Z.F. Chen, D.J. Anderson, and X. Dong. 2009. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell*. 139:1353-1365.
- Liu, Q., H.J. Weng, K.N. Patel, Z. Tang, H. Bai, M. Steinhoff, and X. Dong. 2011. The distinct roles of two GPCRs, MrgprC11 and PAR2, in itch and hyperalgesia. *Sci Signal*. 4:ra45.
- Liu, Y., O. Abdel Samad, L. Zhang, B. Duan, Q. Tong, C. Lopes, R.R. Ji, B.B. Lowell, and Q. Ma. 2010. VGLUT2-dependent glutamate release from nociceptors is required to sense pain and suppress itch. *Neuron*. 68:543-556.
- Lv, L.X., D.Q. Fang, D. Shi, D.Y. Chen, R. Yan, Y.X. Zhu, Y.F. Chen, L. Shao, F.F. Guo, W.R. Wu, A. Li, H.Y. Shi, X.W. Jiang, H.Y. Jiang, Y.H. Xiao, S.S. Zheng, and L.J. Li. 2016. Alterations and correlations of the gut microbiome, metabolism and immunity in patients with primary biliary cirrhosis. *Environ Microbiol*. 18:2272-2286.
- Mansour-Ghanaei, F., A. Taheri, H. Froutan, H. Ghofrani, M. Nasiri-Toosi, A.H. Bagherzadeh, M.J. Farahvash, S. Mirmomen, N. Ebrahimi-Dariani, E. Farhangi, and Z. Pourrasouli. 2006. Effect of oral naltrexone on pruritus in cholestatic patients. *World journal of gastroenterology*. 12:1125-1128.
- Matsumoto, M., A. Aranami, A. Ishige, K. Watanabe, and Y. Benno. 2007. LKM512 yogurt consumption improves the intestinal environment and induces the T-helper type 1 cytokine in adult patients with intractable atopic dermatitis. *Clin Exp Allergy*. 37:358-370.
- Matsumoto, M., T. Ebata, J. Hirooka, R. Hosoya, N. Inoue, S. Itami, K. Tsuji, T. Yaginuma, K. Muramatsu, A. Nakamura, A. Fujita, and T. Nagakura. 2014. Antipruritic effects of the probiotic strain LKM512 in adults with atopic dermatitis. *Ann Allergy Asthma Immunol*. 113:209-216 e207.
- Mayo, M.J., I. Handem, S. Saldana, H. Jacobe, Y. Getachew, and A.J. Rush. 2007. Sertraline as a first-line treatment for cholestatic pruritus. *Hepatology (Baltimore, Md.)*. 45:666-674.

- Mayo, M.J., P.J. Pockros, D. Jones, C.L. Bowlus, C. Levy, I. Patanwala, B. Bacon, V. Luketic, R. Vuppalanchi, S. Medendorp, A. Dorenbaum, C. Kennedy, P. Novak, J. Gu, G. Apostol, and G.M. Hirschfield. 2019. A Randomized, Controlled, Phase 2 Study of Maralixibat in the Treatment of Itching Associated With Primary Biliary Cholangitis. *Hepatology*. 3:365-381.
- McGuire, D. 2017. *clinicaltrials.gov: Safety, Pharmacokinetics and Preliminary Efficacy of Asimadoline in Pruritus Associated With Atopic Dermatitis*. Tioga Pharmaceuticals, Inc.
- McNeil, B., and X. Dong. 2012. Peripheral mechanisms of itch. *Neurosci Bull*. 28:100-110.
- Meixiong, J., C. Vasavda, D. Green, Q. Zheng, L. Qi, S.G. Kwatra, J.P. Hamilton, S.H. Snyder, and X. Dong. 2019a. Identification of a bilirubin receptor that may mediate a component of cholestatic itch. *Elife*. 8.
- Meixiong, J., C. Vasavda, S.H. Snyder, and X. Dong. 2019b. MRGPRX4 is a G protein-coupled receptor activated by bile acids that may contribute to cholestatic pruritus. *Proceedings of the National Academy of Sciences of the United States of America*.
- Meng, L.J., H. Reyes, J. Palma, I. Hernandez, J. Ribalta, and J. Sjovall. 1997. Profiles of bile acids and progesterone metabolites in the urine and serum of women with intrahepatic cholestasis of pregnancy. *Journal of hepatology*. 27:346-357.
- Meyer Zu Heringdorf, D. 2004. Lysophospholipid receptor-dependent and -independent calcium signaling. *J Cell Biochem*. 92:937-948.
- Mishra, S.K., and M.A. Hoon. 2013. The cells and circuitry for itch responses in mice. *Science*. 340:968-971.
- Moolenaar, W.H., and A. Perrakis. 2011. Insights into autotaxin: how to produce and present a lipid mediator. *Nat Rev Mol Cell Biol*. 12:674-679.
- Moore, C., R. Gupta, S.E. Jordt, Y. Chen, and W.B. Liedtke. 2018. Regulation of Pain and Itch by TRP Channels. *Neurosci Bull*. 34:120-142.
- Morita, T., S.P. McClain, L.M. Batia, M. Pellegrino, S.R. Wilson, M.A. Kienzler, K. Lyman, A.S. Olsen, J.F. Wong, C.L. Stucky, R.B. Brem, and D.M. Bautista. 2015. HTR7 Mediates Serotonergic Acute and Chronic Itch. *Neuron*. 87:124-138.
- Murphy, G.M., A. Ross, and B.H. Billing. 1972. Serum bile acids in primary biliary cirrhosis. *Gut*. 13:201-206.
- Nakai, S., T. Masaki, K. Kurokohchi, A. Deguchi, and M. Nishioka. 2000. Combination therapy of bezafibrate and ursodeoxycholic acid in primary biliary cirrhosis: a preliminary study. *Am J Gastroenterol*. 95:326-327.
- Namer, B., R. Carr, L.M. Johaneck, M. Schmelz, H.O. Handwerker, and M. Ringkamp. 2008. Separate peripheral pathways for pruritus in man. *J Neurophysiol*. 100:2062-2069.
- Nishimasu, H., S. Okudaira, K. Hama, E. Mihara, N. Dohmae, A. Inoue, R. Ishitani, J. Takagi, J. Aoki, and O. Nureki. 2011. Crystal structure of autotaxin and insight into GPCR activation by lipid mediators. *Nat Struct Mol Biol*. 18:205-212.
- Oster, Z.H., E.A. Rachmilewitz, E. Moran, and Y. Stein. 1965. Relief of pruritus by cholestyramine in chronic liver disease. *Isr J Med Sci*. 1:599-606.
- Pares, A., L. Caballeria, J. Rodes, M. Bruguera, L. Rodrigo, A. Garcia-Plaza, J. Berenguer, D. Rodriguez-Martinez, J. Mercader, and R. Velicia. 2000. Long-term effects of ursodeoxycholic acid in primary biliary cirrhosis: results of a double-blind controlled multicentric trial. UDCA-Cooperative Group from the Spanish Association for the Study of the Liver. *Journal of hepatology*. 32:561-566.
- Pares, A., L. Cisneros, J.M. Salmeron, L. Caballeria, A. Mas, A. Torras, and J. Rodes. 2004. Extracorporeal albumin dialysis: a procedure for prolonged relief of intractable pruritus in patients with primary biliary cirrhosis. *Am J Gastroenterol*. 99:1105-1110.
- Pares, A., M. Herrera, J. Aviles, M. Sanz, and A. Mas. 2010. Treatment of resistant pruritus from cholestasis with albumin dialysis: combined analysis of patients from three centers. *Journal of hepatology*. 53:307-312.

- Patel, S.P., C. Vasavda, B. Ho, J. Meixiong, X. Dong, and S.G. Kwatra. 2019. Cholestatic pruritus: Emerging mechanisms and therapeutics. *Journal of the American Academy of Dermatology*. 81:1371-1378.
- Pereira, S.P., J.M. Rhodes, B.J. Campbell, D. Kumar, I.M. Bain, G.M. Murphy, and R.H. Dowling. 1998. Biliary lactoferrin concentrations are increased in active inflammatory bowel disease: a factor in the pathogenesis of primary sclerosing cholangitis? *Clin Sci (Lond)*. 95:637-644.
- Pusl, T., G.U. Denk, K.G. Parhofer, and U. Beuers. 2006. Plasma separation and anion adsorption transiently relieve intractable pruritus in primary biliary cirrhosis. *Journal of hepatology*. 45:887-891.
- Quist, R.G., H.T. Ton-Nu, J. Lillienau, A.F. Hofmann, and K.E. Barrett. 1991. Activation of mast cells by bile acids. *Gastroenterology*. 101:446-456.
- Reddy, V.B., A.O. Iuga, S.G. Shimada, R.H. LaMotte, and E.A. Lerner. 2008. Cowhage-evoked itch is mediated by a novel cysteine protease: a ligand of protease-activated receptors. *J Neurosci*. 28:4331-4335.
- Reig, A., P. Sese, and A. Pares. 2018. Effects of Bezafibrate on Outcome and Pruritus in Primary Biliary Cholangitis With Suboptimal Ursodeoxycholic Acid Response. *Am J Gastroenterol*. 113:49-55.
- Reyes, H. 2016. Sulfated progesterone metabolites in the pathogenesis of intrahepatic cholestasis of pregnancy: Another loop in the ascending spiral of medical knowledge. *Hepatology (Baltimore, Md.)*. 63:1080-1082.
- Reyes, H., and J. Sjovall. 2000. Bile acids and progesterone metabolites in intrahepatic cholestasis of pregnancy. *Ann Med*. 32:94-106.
- Robering, J.W., L. Gebhardt, K. Wolf, H. Kuhn, A.E. Kremer, and M.J.M. Fischer. 2019. Lysophosphatidic acid activates satellite glia cells and Schwann cells. *Glia*. 67:999-1012.
- Ross, S.E. 2011. Pain and itch: insights into the neural circuits of aversive somatosensation in health and disease. *Curr Opin Neurobiol*. 21:880-887.
- Ross, S.E., A.R. Mardinly, A.E. McCord, J. Zurawski, S. Cohen, C. Jung, L. Hu, S.I. Mok, A. Shah, E.M. Savner, C. Tolias, R. Corfas, S. Chen, P. Inquimbert, Y. Xu, R.R. McInnes, F.L. Rice, G. Corfas, Q. Ma, C.J. Woolf, and M.E. Greenberg. 2010. Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. *Neuron*. 65:886-898.
- Rust, C., G.H. Sauter, M. Oswald, J. Buttner, G.A. Kullak-Ublick, G. Paumgartner, and U. Beuers. 2000. Effect of cholestyramine on bile acid pattern and synthesis during administration of ursodeoxycholic acid in man. *Eur J Clin Invest*. 30:135-139.
- Schaap, F.G., M. Trauner, and P.L. Jansen. 2014. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol*. 11:55-67.
- Schmelz, M., R. Schmidt, A. Bickel, H.O. Handwerker, and H.E. Torebjork. 1997. Specific C-receptors for itch in human skin. *J Neurosci*. 17:8003-8008.
- Schmelz, M., R. Schmidt, C. Weidner, M. Hilliges, H.E. Torebjork, and H.O. Handwerker. 2003. Chemical response pattern of different classes of C-nociceptors to pruritogens and algogens. *J Neurophysiol*. 89:2441-2448.
- Schworer, H., H. Hartmann, and G. Ramadori. 1995. Relief of cholestatic pruritus by a novel class of drugs: 5-hydroxytryptamine type 3 (5-HT₃) receptor antagonists: effectiveness of ondansetron. *Pain*. 61:33-37.
- Shawcross, D.L., and R. Jalan. 2001. Delayed opioid withdrawal-like reaction in primary biliary cirrhosis following naloxone therapy. *Gastroenterology*. 121:743-744.
- Shelley, W.B., and R.P. Arthur. 1955a. Mucunain, the active pruritogenic proteinase of cowhage. *Science*. 122:469-470.
- Shelley, W.B., and R.P. Arthur. 1955b. Studies on cowhage (*Mucuna pruriens*) and its pruritogenic proteinase, mucunain. *AMA Arch Derm*. 72:399-406.

- Shimada, S.G., K.A. Shimada, and J.G. Collins. 2006. Scratching behavior in mice induced by the proteinase-activated receptor-2 agonist, SLIGRL-NH2. *Eur J Pharmacol.* 530:281-283.
- Snyder, L.M., M.S. Kuzirian, and S.E. Ross. 2016. An Unexpected Role for TRPV4 in Serotonin-Mediated Itch. *The Journal of investigative dermatology.* 136:7-9.
- Soga, F., N. Katoh, T. Inoue, and S. Kishimoto. 2007. Serotonin activates human monocytes and prevents apoptosis. *The Journal of investigative dermatology.* 127:1947-1955.
- Sommer, C. 2004. Serotonin in pain and analgesia: actions in the periphery. *Mol Neurobiol.* 30:117-125.
- Spivey, J.R., R.A. Jorgensen, G.J. Gores, and K.D. Lindor. 1994. Methionine-enkephalin concentrations correlate with stage of disease but not pruritus in patients with primary biliary cirrhosis. *Am J Gastroenterol.* 89:2028-2032.
- Stander, S., B. Bockenholt, F. Schurmeyer-Horst, C. Weishaupt, G. Heuft, T.A. Luger, and G. Schneider. 2009. Treatment of chronic pruritus with the selective serotonin re-uptake inhibitors paroxetine and fluvoxamine: results of an open-labelled, two-arm proof-of-concept study. *Acta Derm Venereol.* 89:45-51.
- Stapelbroek, J.M., K.J. van Erpecum, L.W. Klomp, N.G. Venneman, T.P. Schwartz, G.P. van Berge Henegouwen, J. Devlin, C.M. van Nieuwkerk, A.S. Knisely, and R.H. Houwen. 2006. Nasobiliary drainage induces long-lasting remission in benign recurrent intrahepatic cholestasis. *Hepatology (Baltimore, Md.).* 43:51-53.
- Steinhoff, M., U. Neisius, A. Ikoma, M. Fartasch, G. Heyer, P.S. Skov, T.A. Luger, and M. Schmelz. 2003. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci.* 23:6176-6180.
- Strassburg, C.P. 2010. Hyperbilirubinemia syndromes (Gilbert-Meulengracht, Crigler-Najjar, Dubin-Johnson, and Rotor syndrome). *Best Pract Res Clin Gastroenterol.* 24:555-571.
- Sumida, H., K. Nakamura, K. Yanagida, R. Ohkawa, Y. Asano, T. Kadono, K. Tamaki, K. Igarashi, J. Aoki, S. Sato, S. Ishii, T. Shimizu, and Y. Yatomi. 2013. Decrease in circulating autotaxin by oral administration of prednisolone. *Clinica chimica acta; international journal of clinical chemistry.* 415:74-80.
- Sun, Y.G., and Z.F. Chen. 2007. A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. *Nature.* 448:700-703.
- Sun, Y.G., Z.Q. Zhao, X.L. Meng, J. Yin, X.Y. Liu, and Z.F. Chen. 2009. Cellular basis of itch sensation. *Science.* 325:1531-1534.
- Swain, M.G., R.B. Rothman, H. Xu, J. Vergalla, N.V. Bergasa, and E.A. Jones. 1992. Endogenous opioids accumulate in plasma in a rat model of acute cholestasis. *Gastroenterology.* 103:630-635.
- Tanaka, M., S. Okudaira, Y. Kishi, R. Ohkawa, S. Iseki, M. Ota, S. Noji, Y. Yatomi, J. Aoki, and H. Arai. 2006. Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid. *J Biol Chem.* 281:25822-25830.
- Tandon, P., B.H. Rowe, B. Vandermeer, and V.G. Bain. 2007. The efficacy and safety of bile Acid binding agents, opioid antagonists, or rifampin in the treatment of cholestasis-associated pruritus. *Am J Gastroenterol.* 102:1528-1536.
- Tang, R., Y. Wei, Y. Li, W. Chen, H. Chen, Q. Wang, F. Yang, Q. Miao, X. Xiao, H. Zhang, M. Lian, X. Jiang, J. Zhang, Q. Cao, Z. Fan, M. Wu, D. Qiu, J.Y. Fang, A. Ansari, M.E. Gershwin, and X. Ma. 2018. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut.* 67:534-541.
- Terg, R., E. Coronel, J. Sorda, A.E. Munoz, and J. Findor. 2002. Efficacy and safety of oral naltrexone treatment for pruritus of cholestasis, a crossover, double blind, placebo-controlled study. *Journal of hepatology.* 37:717-722.

- Thebaut, A., D. Debray, and E. Gonzales. 2018. An update on the physiopathology and therapeutic management of cholestatic pruritus in children. *Clin Res Hepatol Gastroenterol.* 42:103-109.
- Thebaut, A., D. Habes, F. Gottrand, C. Rivet, J. Cohen, D. Debray, E. Jacquemin, and E. Gonzales. 2017. Sertraline as an Additional Treatment for Cholestatic Pruritus in Children. *J Pediatr Gastroenterol Nutr.* 64:431-435.
- Thomsen, J.S., M. Sonne, E. Benfeldt, S.B. Jensen, J. Serup, and T. Menne. 2002. Experimental itch in sodium lauryl sulphate-inflamed and normal skin in humans: a randomized, double-blind, placebo-controlled study of histamine and other inducers of itch. *Br J Dermatol.* 146:792-800.
- Thornton, J.R., and M.S. Losowsky. 1988. Opioid peptides and primary biliary cirrhosis. *BMJ.* 297:1501-1504.
- Tian, B., X.L. Wang, Y. Huang, L.H. Chen, R.X. Cheng, F.M. Zhou, R. Guo, J.C. Li, and T. Liu. 2016. Peripheral and spinal 5-HT receptors participate in cholestatic itch and antinociception induced by bile duct ligation in rats. *Scientific reports.* 6:36286.
- Tjandra, K., K.A. Sharkey, and M.G. Swain. 2000. Progressive development of a Th1-type hepatic cytokine profile in rats with experimental cholangitis. *Hepatology (Baltimore, Md.).* 31:280-290.
- Togashi, Y., H. Umeuchi, K. Okano, N. Ando, Y. Yoshizawa, T. Honda, K. Kawamura, T. Endoh, J. Utsumi, J. Kamei, T. Tanaka, and H. Nagase. 2002. Antipruritic activity of the kappa-opioid receptor agonist, TRK-820. *Eur J Pharmacol.* 435:259-264.
- Tokumura, A., E. Majima, Y. Kariya, K. Tominaga, K. Kogure, K. Yasuda, and K. Fukuzawa. 2002. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. *J Biol Chem.* 277:39436-39442.
- Trottier, J., A. Bialek, P. Caron, R.J. Straka, J. Heathcote, P. Milkiewicz, and O. Barbier. 2012. Metabolomic profiling of 17 bile acids in serum from patients with primary biliary cirrhosis and primary sclerosing cholangitis: a pilot study. *Dig Liver Dis.* 44:303-310.
- Ui, H., T. Andoh, J.B. Lee, H. Nojima, and Y. Kuraishi. 2006. Potent pruritogenic action of tryptase mediated by PAR-2 receptor and its involvement in anti-pruritic effect of nafamostat mesilate in mice. *Eur J Pharmacol.* 530:172-178.
- Umeuchi, H., Y. Togashi, T. Honda, K. Nakao, K. Okano, T. Tanaka, and H. Nagase. 2003. Involvement of central mu-opioid system in the scratching behavior in mice, and the suppression of it by the activation of kappa-opioid system. *Eur J Pharmacol.* 477:29-35.
- Umezū-Goto, M., Y. Kishi, A. Taira, K. Hama, N. Dohmae, K. Takio, T. Yamori, G.B. Mills, K. Inoue, J. Aoki, and H. Arai. 2002. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. *J Cell Biol.* 158:227-233.
- Vallejo, M., O. Briz, M.A. Serrano, M.J. Monte, and J.J. Marin. 2006. Potential role of trans-inhibition of the bile salt export pump by progesterone metabolites in the etiopathogenesis of intrahepatic cholestasis of pregnancy. *Journal of hepatology.* 44:1150-1157.
- Van Herpe, F., H.R. Waterham, C.J. Adams, M. Mannens, H. Bikker, F.M. Vaz, and D. Cassiman. 2017. NTCP deficiency and persistently raised bile salts: an adult case. *Journal of inherited metabolic disease.* 40:313-315.
- van Meeteren, L.A., and W.H. Moolenaar. 2007. Regulation and biological activities of the autotaxin-LPA axis. *Prog Lipid Res.* 46:145-160.
- van Meeteren, L.A., P. Ruurs, C. Stortelers, P. Bouwman, M.A. van Rooijen, J.P. Pradere, T.R. Pettit, M.J. Wakelam, J.S. Saulnier-Blache, C.L. Mummery, W.H. Moolenaar, and J. Jonkers. 2006. Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. *Mol Cell Biol.* 26:5015-5022.

- Vaz, F.M., H.H. Huidekoper, and C.C. Paulusma. 2017. Extended Abstract: Deficiency of Sodium Taurocholate Cotransporting Polypeptide (SLC10A1): A New Inborn Error of Metabolism with an Attenuated Phenotype. *Dig Dis.* 35:259-260.
- Vaz, F.M., C.C. Paulusma, H. Huidekoper, M. de Ru, C. Lim, J. Koster, K. Ho-Mok, A.H. Bootsma, A.K. Groen, F.G. Schaap, R.P. Oude Elferink, H.R. Waterham, and R.J. Wanders. 2015. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholanemia without a clear clinical phenotype. *Hepatology (Baltimore, Md.)*. 61:260-267.
- Verkade, H.J., R.J. Thompson, H. Arnell, B. Fischler, P.G. Gillberg, J.P. Mattsson, K. Torfgard, and E. Lindstrom. 2020. Systematic Review and Meta-analysis: Partial External Biliary Diversion in Progressive Familial Intrahepatic Cholestasis. *J Pediatr Gastroenterol Nutr.* 71:176-183.
- Wietholtz, H., H.-U. Marschall, S. Jan, and S. Matern. 1996. Stimulation of bile acid 6 α -hydroxylation by rifampin. *Journal of hepatology.* 24:713-718.
- Wikstrom, B., R. Gellert, S.D. Ladefoged, Y. Danda, M. Akai, K. Ide, M. Ogasawara, Y. Kawashima, K. Ueno, A. Mori, and Y. Ueno. 2005. Kappa-opioid system in uremic pruritus: multicenter, randomized, double-blind, placebo-controlled clinical studies. *J Am Soc Nephrol.* 16:3742-3747.
- Wilson, S.R., K.A. Gerhold, A. Bifolck-Fisher, Q. Liu, K.N. Patel, X. Dong, and D.M. Bautista. 2011. TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nat Neurosci.* 14:595-602.
- Woolbright, B.L., K. Dorko, D.J. Antoine, J.I. Clarke, P. Gholami, F. Li, S.C. Kumer, T.M. Schmitt, J. Forster, F. Fan, R.E. Jenkins, B.K. Park, B. Hagenbuch, M. Olyae, and H. Jaeschke. 2015. Bile acid-induced necrosis in primary human hepatocytes and in patients with obstructive cholestasis. *Toxicol Appl Pharmacol.* 283:168-177.
- Wunsch, E., M. Krawczyk, M. Milkiewicz, J. Trottier, O. Barbier, M.F. Neurath, F. Lammert, A.E. Kremer, and P. Milkiewicz. 2016. Serum Autotaxin is a Marker of the Severity of Liver Injury and Overall Survival in Patients with Cholestatic Liver Diseases. *Scientific reports.* 6:30847.
- Xie, Z., and H. Hu. 2018. TRP Channels as Drug Targets to Relieve Itch. *Pharmaceuticals (Basel, Switzerland)*. 11.
- Xu, J., G. Lee, H. Wang, J.M. Vierling, and J.J. Maher. 2004. Limited role for CXC chemokines in the pathogenesis of alpha-naphthylisothiocyanate-induced liver injury. *Am J Physiol Gastrointest Liver Physiol.* 287:G734-741.
- Yagi, M., A. Tanaka, T. Namisaki, A. Takahashi, M. Abe, A. Honda, Y. Matsuzaki, H. Ohira, H. Yoshiji, H. Takikawa, and P.B.C.S.G. Japan. 2018. Is patient-reported outcome improved by nalfurafine hydrochloride in patients with primary biliary cholangitis and refractory pruritus? A post-marketing, single-arm, prospective study. *J Gastroenterol.* 53:1151-1158.
- Yamaguchi, T., T. Nagasawa, M. Satoh, and Y. Kuraishi. 1999. Itch-associated response induced by intradermal serotonin through 5-HT₂ receptors in mice. *Neurosci Res.* 35:77-83.
- Yamazaki, T., S. Joshita, T. Umemura, Y. Usami, A. Sugiura, N. Fujimori, S. Shibata, Y. Ichikawa, M. Komatsu, A. Matsumoto, K. Igarashi, and E. Tanaka. 2017. Association of Serum Autotaxin Levels with Liver Fibrosis in Patients with Chronic Hepatitis C. *Scientific reports.* 7:46705.
- Yosipovitch, G., and J.D. Bernhard. 2013. Clinical practice. Chronic pruritus. *N Engl J Med.* 368:1625-1634.
- Yosipovitch, G., J.D. Rosen, and T. Hashimoto. 2018. Itch: From mechanism to (novel) therapeutic approaches. *J Allergy Clin Immunol.* 142:1375-1390.
- Yu, H., T. Zhao, S. Liu, Q. Wu, O. Johnson, Z. Wu, Z. Zhuang, Y. Shi, L. Peng, R. He, Y. Yang, J. Sun, X. Wang, H. Xu, Z. Zeng, P. Zou, X. Lei, W. Luo, and Y. Li. 2019. MRGPRX4 is a bile acid receptor for human cholestatic itch. *Elife.* 8.

- Zhao, J., A. Munanairi, X.Y. Liu, J. Zhang, L. Hu, M. Hu, D. Bu, L. Liu, Z. Xie, B.S. Kim, Y. Yang, and Z.F. Chen. 2020. PAR2 Mediates Itch via TRPV3 Signaling in Keratinocytes. *The Journal of investigative dermatology*. 140:1524-1532.
- Zhao, Z.Q., X.Y. Liu, J. Jeffry, W.K. Karunaratne, J.L. Li, A. Munanairi, X.Y. Zhou, H. Li, Y.G. Sun, L. Wan, Z.Y. Wu, S. Kim, F.Q. Huo, P. Mo, D.M. Barry, C.K. Zhang, J.Y. Kim, N. Gautam, K.J. Renner, Y.Q. Li, and Z.F. Chen. 2014. Descending control of itch transmission by the serotonergic system via 5-HT1A-facilitated GRP-GRPR signaling. *Neuron*. 84:821-834.