Molecular mechanisms of pruritus in cholestasis
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Citation for published version (APA):

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Receptors, cells and circuits involved in pruritus of systemic disorders

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ABSTRACT

Pruritus is a sensory phenomenon accompanying a broad range of systemic disorders including hematologic and lymphoproliferative disorders, metabolic and endocrine diseases, solid tumours, and infectious diseases. The molecular mechanisms involved in itch sensation remain enigmatic in most of these diseases. However, from studies in patients and animal models a large number of mediators and receptors responsible for scratching behaviour have been identified in recent years. New insights in the interplay between neuronal and non-neuronal cells involved in the initiation, modulation and sensitization of itch sensation have been acquired. This review highlights the current knowledge of the molecular mechanism of pruritus in systemic disorders and summarizes the signalling pathways of biogenic amines, neuropeptides, proteases, eicosanoids, cytokines, opioids, endocannabinoids, neurotrophins, phospholipids and other signalling molecules participating in pruritus. Furthermore, recent experimental and clinical findings focusing on the pathogenesis and actual treatment of pruritus in cholestatic liver disease are highlighted. Evidence-based therapeutic recommendations including the use of anion exchange resins cholestyramine, colestipol, and colesevelam, the microsomal enzyme inducer rifampicin, the opioid antagonists naltrexone and naloxone, and the selective serotonin receptor reuptake inhibitor sertraline are provided.
INTRODUCTION

Acute pruritus serves as an alarm signal to protect the body against potentially harmful environmental threats such as parasites, noxious plants or other irritants. The scratch response helps to remove these harmful agents from the skin and diminishes itch sensation. These acute forms of pruritus are mainly mediated by histamine-responsive sensory neurons in the skin that are relatively insensitive to mechanical pain stimuli but also respond to noxious chemicals such as capsaicin. Chronic pruritus can be a seriously debilitating symptom accompanying various cutaneous and systemic disorders, but may also be caused by drugs such as the anti-malaria drug chloroquine or the volume expander hydroxyethyl starch. As antihistamines do not improve itching in most of these conditions, it is likely that itch sensation is mediated via histamine-independent pathways. Recently discovered receptors involved in itch signalling of rodents such as the Mas-related G protein-coupled receptors (Mrg) for chloroquine, BAM8-22 and β-alanine, the μ-opioid receptor 1D for morphine-induced pruritus, endothelin-A-receptor for endothelin-1, as well as the interleukin-13 and interleukin-31 receptor have been shown to mediate itch sensation in a histamine-independent fashion. Some of these and other receptors are G protein-coupled to phospholipase C (PLC), among which histamine-1- and serotonin-(5-HT2)-receptors activate specifically the beta 3 isoform (PLCβ3). Formation of intracellular signalling molecules such as diacylglycerol (DAG) and inositol-3-phosphate causes intracellular calcium release and activation of protein kinase C (PKC) resulting in opening of transient receptor potential (TRP) receptors such as the vanilloid 1 receptor (TRPV1), TRPV3, or ankyrin 1 channel (TRPA1) which is required for neuronal excitation (Figures 1-4). These primary sensory neurons signal to the dorsal horn of the spinal cord where secondary neurons are activated by release of glutamate and the neuropeptide natriuretic polypeptide b (Nppb). These secondary, Nppb receptor expressing, neurons are suggested to release gastrin releasing peptide (GRP) which activates the GRP receptor of a third neuron in the spinal cord. Besides the GRP-receptor also the neuromedin B-receptor has been shown to be responsible for mediating itch signals. Ablation of either the Nppb- or GRP-receptor expressing neurons by intrathecal application of a toxin bound to the respective signalling molecule largely
abolished scratching behaviour after intradermal application of various pruritogens.\textsuperscript{14,16} Noteworthy, nociceptive (pain) stimuli were unaltered by the ablation of these neurons, indicating that a selective itch pathway exists on spinal cord level.\textsuperscript{14,16} However, pain and itch signalling are closely intertwined processes: activation of pain neurons inhibits itch sensation, e.g. by scratching, cooling or heating of the skin,\textsuperscript{19,20} whereas antinociception can cause itch sensation, e.g. by epidural or intrathecal application of opioids or anaesthetics (Figure 1).\textsuperscript{21-23}

Figure 1: Neurotransmitters, receptors and channels that play a role in itch and pain signalling. Simplified scheme of pain and itch signalling pathways from the peripheral to the central nervous system and their interaction. Itch- and pain-causing molecules bind to specific receptors on sensory nerve endings in the epidermis or dermis. Among the established receptors for itch signalling are histamine (H1, H4), serotonin (5-HT2), Mas-gene related G protein coupled receptors (MrgA3, MrgC11, MrgD), endothelin (ET-A), protease activated receptor (PAR4), toll-like receptor 3 (TLR3) and 7 (TLR7), and heterodimeric receptor consisting of the IL-31 receptor α (IL-31RA) and the oncostatin M receptor (OSMR). These neurons also express transient receptor potential (TRP) receptors such as TRPV1 and TRPA1. Pruritus may also be initiated or potentiated by lysophosphatidic acid (LPA) receptors. Synaptic signal transmission from the peripheral sensory neuron to the secondary neuron in the dorsal horn of the spinal cord is mediated by glutamate (Glut) and natriuretic polypeptide b (Nppb). Gastrin-releasing peptide (GRP) and glutamate may be involved in signal transmission to the tertiary neuron. The neuronal itch signalling pathway is under inhibitory control of the pain signals (as indicated by the Bhlhb5- and Prdm8-expressing interneurons). Pain sensation is similarly perceived by receptors on peripheral sensory neurons including neurokinin-1 for substance P or protease-activated receptor 2 (PAR-2) for proteases. Synaptic signal transmission from the peripheral sensory neuron to the secondary pain neurons and interneurons in the dorsal horn of the spinal cord is presumably mediated by glutamate (Glut), substance P (SP), and calcitonin-gene related peptide (CGRP). Putative signalling pathway of chloroquine in itch neurons. For abbreviations see text.
Thus, the itch circuitry was assumed to stand under a tonic inhibitory control of mechano-sensitive neurons. This hypothesis was recently strengthened by the observation of spontaneous intense scratching behaviour in mice lacking certain inhibitory, Bhlhb5- and Prdm8-expressing interneurons. These interneurons are believed to be activated by glutamate, as the deletion of the glutamate transporter VGLUT2 caused increased spontaneous as well as induced scratching activity after application of pruritogens (Figure 1). In spite of this growing knowledge of receptors and pathways responsible for itch signalling in mice, rats and other species, the responsible ligands and receptors for itch sensation in human beings remain unidentified for most disorders associated with chronic pruritus.

Pruritus represents one of the most prominent clinical features in a wide range of systemic disorders including (i) hematologic and lymphoproliferative disorders such as polycythemia vera, essential thrombocytosis, primary myelofibrosis, and lymphoma, (ii) metabolic and endocrine diseases such as hepatobiliary diseases, chronic renal disorders, thyroid and parathyroid disorders and diabetes mellitus, (iii) solid tumours, (iv) infectious diseases, and (v) pruritus in the elderly.

This review summarizes the current knowledge on molecular mechanisms of pruritus associated with systemic disorders. Furthermore, the growing insight into receptors and pathways responsible for itch signalling is outlined in detail.

**MOLECULAR SIGNALLING PATHWAYS OF PRURITOGENS**

**Biogenic amines**

- **Histamine**

The biogenic amine histamine which is derived from the amino acid histidine, is certainly the classic itch mediator and best studied pruritogen. Intradermal application of histamine by either iontophoresis or intradermal injection causes itching after a characteristic latency of up to one minute which is accompanied by a wheal and surrounding flare. Noteworthy,
the location of application is important whether histamine acts as a pruritoceptive or nociceptive compound. Superficial cutaneous application of histamine induces pruritus whereas a deeper subcutaneous injection causes mainly pain. This difference is also clinically well known: histamine release from mast cells in the dermis causes itching urticaria characterized by skin rash and wheals. In contrast, histamine liberated in subcutis, mucosa or submucosal tissue provokes angioedema being characterized by swelling of the affected tissue. This swelling is commonly painful, at least hyperalgesic and not itchy.

Histamine-induced itch is caused by direct stimulation of histamine-1-receptors (H1-receptors) on sensory nerve endings. The H1-receptor is linked to a G-protein (Gq), which – upon binding of histamine – activates phospholipase C β3 (PLCβ3), which in turn cleaves phosphatidylinositol-4,5-bisphosphate (PIP2) into the second messengers diacylglycerol (DAG) and inositol-triphosphate (IP3) (see fig. 2 for schematic representation). DAG activates proteinkinase Cε (PKCε) which phosphorylates and thereby opens the transient receptor potential vanilloid receptor 1 (TRPV1). Although controversially discussed, removal of PIP2 has been shown to disinhibit TRPV1. TRPV1 is activated by several other signaling molecules, including kinases (PKC, PKA and CamKII) and desensitized by Ca²⁺-dependent phosphatase 2B (calcineurin) that are brought into spatial proximity by the AKAP scaffolding protein. Activation of TRPV1 leads to channel opening which allows passage of the positively charged ions sodium, potassium and calcium resulting in depolarization. Thereby voltage-dependent sodium channels are activated generating action potentials along the nerve fibre which lead to sensation of itch. Histamine-induced pruritus is partially mediated by activation of TRPV1 and requires phosphoinositide-interacting regulator of transient receptor potential channels (PIRT), a membrane protein modulating TRPV1 function. PIRT is required for a PIP2-dependent activation of TRPV1.
Neuronal circuits in pruritus of systemic disorders

Figure 2: Putative signalling pathway of histamine in itch neurons. For abbreviations see text.

In addition, activation of TRPV1 in histamine-induced scratching behaviour relates to activation of phospholipase A2 and lipooxygenase generated products including 12-hydroxyeicosatetraenoic acid (12-HETE). TRPV1 can be activated by increased temperature (>43°C), protons and the prototypic agent capsaicin (red hot chili pepper) but also by a host of endogenous and exogenous compounds like vanilloids, phorbol esters, camphor, endogenous lipids such as anandamide and 12-HETE or lipid metabolic products such as LPA.

Wheal and flare are typical skin alterations seen in histamine-mediated pruritus which are mainly due to secondary release of vasoactive substances such as calcitonin gene-related peptide (CGRP) and substance P from axon collaterals. The wheal is mediated by histamine itself and substance P binding to NK1-receptors on endothelial cells, whereas the flare reaction is mediated by CGRP. Wheal and flare are also observed in patients with urticaria, cutaneous mastocytosis, acute drug rashes and allergic skin reactions due to contact with certain parasites, insects or plants. Antagonists of the H1-receptor, so called H1-antihistamines, effectively alleviate pruritus in these conditions. However, these typical skin changes are not observed in most patients with chronic pruritus due to systemic disorders and, accordingly, antagonists of the H1-receptor are mostly ineffective in these conditions.
patients. Furthermore, tachyphylaxis is observed after repeated application of histamine to the skin, indicating that histamine is less likely to be responsible for chronic pruritus.  

Although histamine levels have been reported to be slightly increased in systemic diseases including cholestasis and uraemia, other mediators seem to cause pruritus in these disorders.

For decades the histamine receptors H₁ and H₂ were known and have been studied. Using specific agonists it could be shown that itch is caused by activation of H₁-receptors, whereas H₂-receptor agonists could neither induce pruritus nor potentiate the pruritic effect of H₁-receptor agonists. Notably, Davies and Greaves demonstrated almost 30 years ago that the itch threshold for a specific H₁-receptor agonist was consistently higher than for histamine itself even in the presence of a H₂-receptor antagonist. The authors suggested that histamine-induced pruritus was mediated via H₁-receptors and partly via an additional non-H₂-receptor. In recent years, two further histamine receptors, H₃-receptor and H₄-receptor, have been discovered. The H₃-receptor is mainly expressed in the peripheral and central nervous system, whereas H₄-receptors are found on sensory neurons, keratinocytes, mast cells and CD4⁺ T-cells. The activation of H₃-receptor seems to suppress itch sensation, as receptor antagonists have been shown to cause scratching behaviour in mice. H₄-receptors could indeed represent the subclass of histamine receptors, Davies and Greaves have speculated on. A H₄-receptor agonist (clobenpropit) caused scratching behaviour in Balb/c mice in a dose-dependent manner which was not inhibited by H₁- or H₂-receptor antagonists. Combining H₁- and H₄-receptor antagonists has been reported to be more effective than either one alone, indicating that these receptors have non-redundant roles in itch sensation. Pharmacological inhibition of H₄-receptors by the specific inhibitor JNJ-7777120 diminished histamine-induced scratching behaviour more effectively than common H₁-receptor antagonists. Moreover, this compound reduced scratch activity, diminished lymphocyte proliferation and attenuated Th2-specific cytokine release in an animal model of atopic dermatitis and diminished existing airway inflammation in a mouse model of asthma. If H₄-receptor antagonists prove their beneficial effects in clinical trials, they represent a promising novel class of antihistamines.
Neuronal circuits in pruritus of systemic disorders

- **Serotonin**

The biogenic amine serotonin (= 5-hydroxytryptamine) which is derived from the amino acid tryptophan is a neurotransmitter in the central nervous system and present in high concentrations in platelets. Upon intradermal injection serotonin provokes itching albeit to a much lower extent than histamine, and even high concentrations did not induce pruritus but burning pain in some healthy subjects. Notably, a combined injection of serotonin and prostaglandin E2 markedly increased itch severity and caused itching even in those subjects not responding to serotonin alone. In humans, intradermal application of serotonin had a shorter latency of inducing itch sensation than histamine which may indicate a direct effect of serotonin on sensory neurons. Intradermal application of serotonin causes scratching behaviour in mice by activation of metabotropic Gq/G11-protein coupled 5-HT2 receptors, which is in line with the observation that α-methyl-serotonin, a selective 5-HT2 receptor agonist, but not 5-HT1 or 5-HT3 receptor agonists, cause a similar scratching behaviour. In line with this, serotonin-mediated scratching behaviour was not altered in 5-HT3 receptor deficient mice. Similar to histamine, serotonin-induced scratching behaviour requires activation of phospholipase Cβ3 (PLCβ3) and TRPV1 as shown in knock-out animals (fig. 3). Recently, endocannabinoids have been reported to attenuate serotonin-induced scratching behaviour of spinally innervated skin, whereas that of trigeminally innervated skin was augmented. Peripheral endocannabinoids seem to have opposed effects on itch-related scratching behaviours depending on the location of the affected skin. Furthermore, serotonin-induced scratching behaviour in mice may in part involve the central opioid neurotransmitter system as it is diminished by the µ-opioid-antagonist naloxone which has antipruritic effects also in other murine models of acute pruritus. An interaction between the opioidergic and serotoninergic system was suggested by the observation that the specific 5-HT3 receptor antagonist ondansentron abolished pruritus caused by spinally administered opioids. However, in systemic disorders associated with pruritus including chronic liver disorders and uraemia, ondansentron was of no or only of minimal benefit in randomized, placebo-controlled trials. Conversely, the selective serotonin re-uptake inhibitors (SSRI) sertraline, paroxetine and fluvoxamine have been reported to exert some beneficial anti-pruritic effects in chronic liver disorders, atopic dermatitis, lymphoma and solid tumours presumably via modulation of neurotransmitter levels in the central
nervous system. Similarly, the selective norepinephrine re-uptake inhibitor (SNRI) mirtazapine could reduce itch severity in uncontrolled case series.\textsuperscript{70,71} Randomized, placebo-controlled trials are warranted to strengthen these positive observations.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Putative signalling pathway of serotonin in itch neurons. For abbreviations see text.}
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### Neuropeptides

- **Substance P**

The undecapeptide substance P is an extensively studied neuropeptide which is an important signalling co-transmitter of afferent neurons in the peripheral and central nervous system.\textsuperscript{72} Upon activation of sensory neurons located in the skin, substance P is released which indirectly causes itch sensation – only at high, presumably unphysiological concentrations – by activation of neurokinin-receptors on non-neuronal cells located in the skin such as keratinocytes and mast cells.\textsuperscript{1,73} Indeed, intradermal injection of substance P in humans activated mast cells to secrete histamine which was accompanied by a wheal and flare reaction as observed after histamine injections.\textsuperscript{74-77} These responses were inhibited by pre-treatment of antihistamines or the histamine liberator compound 48/80, which depletes local histamine stores, suggesting that substance P-induced pruritus is mast cell-
Mast cells being activated by substance P can additionally release further inflammatory mediators such as leukotriene B4, prostaglandin D2, and TNF-α which induce further release of substance P from sensory nerve endings and in turn lead to stronger mast cell activation. On the other hand, tryptase and chymase being released from mast cells are capable to degrade substance P. Beside mast cells substance P may trigger the release of pruritogenic compounds from other cell types such as keratinocytes, endothelial cells, and immune cells.

In mice, intradermal injection of substance P caused a similar scratching response in mast-cell deficient mice as compared to wild-type mice. The contention that substance P-mediated itch is mast cell dependent in humans is questioned by the observation that scratching behaviour was reduced in wild-type and mast cell deficient mice to a similar extent by pre-treatment with compound 48/80. Additionally, it could be shown that scratch responses were mediated via the neurokinin-1-receptor (NK1-receptor – although not present on DRGs), but not NK2- or NK3-receptor. Notably, substance P induced scratching behaviour in mice was strongly reduced by glycyrrhetinic acid, a pentacyclic triterpenoid derivative obtained by hydrolysis of glycyrrhizic acid, possibly via inhibition of leukotriene B4 synthesis in the skin. Besides its release from sensory neurons substance P was shown to play a central role in signalling of itch sensations in the spinal cord of rats via the NK1-receptor. These second order, postsynaptic neurons undergo facilitation of their synaptic transmission (“spinal /central sensitization”) which may contribute to alloknesis (light mechanical stimuli, brushing or stroking evoking itch). In mice lacking TRPA1 the indirect activation of sensory neurons by substance P was hampered and scratching activity to substance P abolished. TRPA1 is a member of the TRP-family (which also includes TRPV1), that is activated by cold (<17 °C) as well as by a plethora of chemical compounds such as isothiocyanates (the pungent compounds in mustard oil, wasabi, and horseradish), methyl salicylate (in winter green oil), cinnamaldehyde (in cinnamon), allicin and diallyl disulphide (in garlic), acrolein (an irritant in wood fire and tobacco smoke) and Δ9 tetra-hydrocannabinol (Δ9THC, the psychoactive compound in marijuana) and many others. Activation of TRPA1 generates pain signals but recent evidence suggests that it also plays a role in transduction of itch, (also see below).
In patients with atopic dermatitis increased serum levels of substance P were described, which correlated with the reported itch intensity. Moreover, lesional skin of patients with atopic dermatitis and prurigo nodularis is characterized by increased substance P positive sensory neurons. Recently, the antiemetic NK1-receptor antagonist aprepitant was shown in uncontrolled case series to effectively reduce pruritus in patients with various dermatological and some systemic disorders, Sézary syndrome, solid tumours, and pruritus due to the epidermal growth factor inhibitor erlotinib. These very promising results warrant confirmation in randomized, placebo-controlled trials.

- **Calcitonin-gene related peptide**

The polypeptide calcitonin-gene related peptide (CGRP) is abundantly expressed in somatic and vagal sensory neurons but may also be expressed in Langerhans cells or keratinocytes. In contrast to substance P which indirectly causes pruritus after intradermal injection, CGRP is controversially discussed as pruritogen. CGRP modulates itch sensation and inflammation rather than directly causing pruritus. Intradermal injected CGRP rather had an itch-inhibitory effect and prolonged the itch latency of subsequently injected substance P, whereas no effect was observed on wheal and flare reaction.

In patients with atopic dermatitis, prurigo nodularis and nummular eczema an increased number of CGRP positive nerve fibres has been described in affected skin areas. Furthermore, CGRP increased IL-13 levels in cutaneous lymphocyte-associated antigen positive T-cells (CDA+ T-cells) from patients with atopic dermatitis but not healthy controls. The more IL-13 was released from T-cells the higher were total IgE levels and percentage of inflamed skin. Thus, CGRP seems to modulate CLA+ T-cells towards a TH2 pattern and may contribute to exacerbating clinical symptoms in itchy skin disorders.

The function of peptidergic CGRPα-expressing sensory neurons could recently be revealed by genetic ablation of these sensory neurons. Mice lacking CGRPα+ sensory neurons exhibited an attenuated histamine- and chloroquine-induced scratching behaviour while that to β-alanine was unaltered. These mice also showed an impaired sensitivity to
noxious heat and capsaicin, but enhanced behaviour to cold stimuli including activation of TRPM8, indicating that CGRPα+ sensory neurons encode for heat and certain itch stimuli and tonically cross-inhibit cold-responsive spinal neurons.104

- **Endothelin-1**

Endothelin-1 (ET-1) is a polypeptide which is synthesized by various cell types, including endothelial cells, vascular smooth muscle cells, keratinocytes and inflammatory cells.105,106 Of note, at lower concentrations ET-1 exerts vasodilatory effects presumably by the release of nitric oxide and prostacyclin, and only higher levels induce a powerful vasoconstriction.105 ET-1 is also capable of directly activating mast cells, keratinocytes, subsets of sensory neurons and glia cells.107,108 Subcutaneous injection of ET-1 caused nociceptive behaviours in animals109-111 and induced burning pain in human beings.112 However, ET-1 has also been reported to cause scratch responses in mice11,111,113,114 as well as itching associated with a burning character in humans.115,116 A well-performed microneurography study in human volunteers proved that ET-1 activated and sensitized mechanosensitive C fibres concentration-dependently causing itching and pain, which was only in part mediated by histamine release.116 Intradermal application of ET-1 caused a scratching response mainly via the activation of endothelin-A-receptors (ETA-receptor, present on mast cells and sensory neurons) as shown by the use of specific agonists and antagonists in mice.7,113,114 ET-1 belongs to the most potent pruritogens as it elicits scratching behaviour in animals in the picomolar range, whereas other pruritogens require micromolar concentration, indicating that endothelin-1 is likely to directly act on its cognate receptor on sensory neurons.11 The ETA-receptor is thought to predominantly act via a Gq/11-PCR stimulating phospholipase C, but may also activate phospholipases A2 and D.117 ET-1-induced scratching behaviour was virtually abolished in mice that had lost TRPV1-expressing neurons11 (see fig. 4 for schematic representation). A recent study suggested that inhibition of TRPA1 would increase endothelin-induced scratch response.118 Further analyses in knock-out animals are warranted to reveal the role of TRPA1 in scratch response induced by ET-1 and other mediators.
Figure 4: Putative signalling pathway of endothelin in itch neurons. For abbreviations see text.

Proteases

More than 50 years ago Shelley and Arthur reported about spicules of the tropical legume Mucuna pruriens (cowhage) that caused itching after rubbing into the skin without a visible wheal or flare reaction.\textsuperscript{119-121} Thus, itching was believed to be independent of histamine which was later underlined by the fact that cowhage still produced intense pruritus in skin rendered tachyphylactic to histamine.\textsuperscript{41} The enzymatic action of a protease isolated from the spiculae, which was named mucunain by Shelley and Arthur, was believed to cause histamine-independent pruritus.\textsuperscript{120} Shortly afterwards various endogenous proteases such as trypsin, chymotrypsin, and, fibrolysin as well as exogenous proteases including papain and streptokinase were reported to induce pruritus.\textsuperscript{36,122} In recent years it was uncovered that the responsible receptors for histamine-independent itch signalling by proteases were the G protein-coupled receptors called protease-activated receptor 2 (PAR2) and 4 (PAR4).\textsuperscript{123,124} It was shown that the activation of PAR2 depends on proteolytic cleavage of the N-terminal extracellular part of the receptor by which a peptide is released that activates the receptor as a tethered ligand. The free six amino acid residue Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL-NH$_2$), which is widely used to induce nonhistaminergic, protease-mediated itching, was found to cause scratching behaviour by activating PAR2.\textsuperscript{125} Thus, it was concluded that PAR2 may be activated by various endogenous (trypsin, tryptase, kallikreins, cathepsin S,
etc.) and exogenous proteases (house-dust mite, bacteria, etc.)\textsuperscript{126,127} However, Liu and colleagues could recently clearly show that the scratch response caused by the peptide SLIGRL is due to activation of Mas-related G protein-coupled receptor C11 (MrgC11; see also later in this review) rather than PAR2\textsuperscript{128} Hence, the target of the liberated peptide does not seem to be PAR2 itself but rather the MrgC11 receptor. Notably, the one amino acid shorter peptide SLIGR specifically activated PAR2 which caused thermal pain hypersensitivity but not a scratch response in mice. Furthermore, scratching behaviour after intradermal application of trypsin was not reduced in PAR2\textsuperscript{-/-} mice,\textsuperscript{128} indicating that proteases cause an itch-scratch response by a yet to be determined receptor which could be e.g. PAR4. Another recent study indicated that the pruritic effect of proteases may at least partly be mediated by release of endothelin-1 from keratinocytes.\textsuperscript{129}

PAR2 was shown to be expressed on primary spinal afferent neurons which released the neuropeptides substance P and CGRP upon stimulation with tryptase.\textsuperscript{130,131} Binding of the ligand to PAR2 activates a phospholipase C (but not PLC\textsubscript{β3}) which in turn cleaves PIP2 into DAG and IP\textsubscript{3}.\textsuperscript{31} DAG activates protein kinase C (PKC) which phosphorylates and activates the transient receptor potential vanilloid receptor 1 (TRPV1).\textsuperscript{132,133} Of note, PAR2 activation has been shown to sensitize TRPV1 in various states of pain sensation,\textsuperscript{132-134} instead of playing a role in itch signalling and sensitization.\textsuperscript{128}

In humans, the endogenous PAR2 activator tryptase was found to be elevated in skin of atopic dermatitis patients, whereas histamine levels were not different from healthy controls.\textsuperscript{123} Furthermore, PAR2 expression on afferent sensory neurons was markedly increased in skin of atopic dermatitis patients compared to healthy controls.\textsuperscript{123} PAR2 expression on keratinocytes was similarly up-regulated in the epidermis of atopic dermatitis patients with the highest expression in skin lesions.\textsuperscript{135} Furthermore, specific PAR2 agonists provoked enhanced and prolonged itch in such patients upon intralesional application.\textsuperscript{123} These observations stand in contrast to the findings in mice by Liu and colleagues (mentioned above)\textsuperscript{128} and could be due to species differences in effective ligands or a release of pruritogens by activation of PAR2 in keratinocytes (and potentially other skin cells) in patients with atopic dermatitis.
Eicosanoids

- **Prostaglandins**

Prostaglandins are synthesized by the enzyme cyclooxygenase (COX) from arachidonic acid, which is liberated from phospholipids by the action of phospholipase A_2_. Prostaglandins have been well-studied in inflammation and pain, however, the role in itch sensation is rather unclear. Prostaglandins are regarded as modulators of pruritus rather than being direct pruritogens. Intradermally injected prostaglandin E_1_ (PGE_1_), E_2_ (PGE_2_) and H_2_ had no or only a weak pruritogenic effect, whereas they markedly enhanced histamine-induced pruritus.\(^{136,137}\) The effect of PGE_2_ was not altered by pre-treatment with the H_1_-antihistamine clemastine suggesting that PGE_2_ acts downstream of the histamine receptor, potentiates a non-histaminergic itch pathway or acts via other histamine-receptors.\(^{138}\) Similarly, PGE_1_ could potentiate protease-induced itching of papain\(^{139}\) and PGE_2_ augmented serotonin-induced pruritus in healthy controls.\(^{56}\) The weak direct pruritic effect of PGE_2_ was comparable in healthy controls and patients with atopic dermatitis.\(^{140}\) Non-steroidal anti-inflammatory drugs (= NSAID = COX-inhibitors) which are widely prescribed suppress the synthesis of prostaglandins. In systemic disorders with pruritus, however, NSAID have only been reported to attenuate itching in patients with polycythemia vera and HIV infection.\(^{141-143}\) Thus, the impact of prostaglandins on pathogenesis of pruritus seems to be limited.

In mice, prostaglandin D_2_ suppressed IgE-mediated scratch response of ovalbumine-sensitized mice which was explained by reduced histamine release from mast cells.\(^{144}\) The increased scratching behaviour induced by a selective COX-1-inhibitor in NC/Nga mice which spontaneously develop eczematous atopic dermatitis-like skin lesions was explained by the diminished PGD_2_-synthesis.\(^{145}\) Indeed, when PGD_2_ was applied topically to these mice the COX-1-inhibitor induced scratching behaviour was suppressed.\(^{145}\) Inhibition of itch sensation by PGD_2_ was shown to be mediated via the prostanoid D receptor 1 (PD1) and activation of the p38-MAPK pathway.\(^{146}\) In humans, however, PGD_2_ did neither diminish histamine-induced pruritus nor the accompanying wheal and flare reaction.\(^{147}\)
Neuronal circuits in pruritus of systemic disorders

- **Leukotrienes**

Leukotrienes also belong to the group of eicosanoids, but are synthesized from arachidonic acid by the enzyme lipooxygenase (LO). Leukotriene B\textsubscript{4} (LTB\textsubscript{4}) has been reported to induce scratching behaviour in mice.\textsuperscript{148} Furthermore, substance P-induced scratching may in part be mediated by release of LTB\textsubscript{4} from keratinocytes.\textsuperscript{149} Similarly, sphingosine phosphorylcholine (SPC)-induced pruritus was in part explained by synthesis and secretion of LTB\textsubscript{4} from keratinocytes, as scratching behaviour was diminished by the 5-lipoxygenase inhibitor zileuton and the LTB\textsubscript{4}-antagonist ONO-4057.\textsuperscript{150} Recently, the MrgC11-agonist SLIRGL-NH\textsubscript{2} was shown to increase synthesis and release of LTB\textsubscript{4} and PGE\textsubscript{2} from keratinocytes \textit{in vitro} and \textit{in vivo}.\textsuperscript{151} The authors observed that topically applied tacrolimus attenuated SLIRGL-NH\textsubscript{2}-induced scratch responses in mice and suggested that this effect was due to a diminished synthesis and/or release of LTB\textsubscript{4} and PGE\textsubscript{2} from keratinocytes.\textsuperscript{151} The underlying mechanisms behind this observation remain unclear and it should be mentioned that tacrolimus inhibits calcineurin (phosphatase 2B) which dephosphorylates and desensitizes TRPV1.

In contrast to mice, intradermally injected leukotrienes including LTB\textsubscript{4}, LTC\textsubscript{4}, LTD\textsubscript{4} and LTE\textsubscript{4} did not evoke itch in healthy human subjects.\textsuperscript{152,153} However, LTB\textsubscript{4}-levels were increased in lesional skin of patients with atopic dermatitis and psoriasis.\textsuperscript{154,155} Notably, the leukotriene antagonist montelukast attenuated pruritus in a randomized, placebo-controlled trial in atopic dermatitis patients\textsuperscript{156} and in a placebo-controlled, crossover trial of uremic patients on haemodialysis.\textsuperscript{157} Leukotrienes might therefore modulate itch severity in patients with atopic dermatitis and uraemia.

**Cytokines**

Certain cytokines released from leukocytes are capable of eliciting itch sensations. Intradermal injection of interleukin-2 (IL-2) caused itching and erythema in both, healthy subjects and atopic dermatitis patients, which lasted for 48–72 hours.\textsuperscript{158} Intravenous IL-2 which has been used to treat metastatic carcinoma often induced severe pruritus.\textsuperscript{159-161} In
serum of haemodialysis patients with pruritus enhanced levels of IL-2 but not IL-4 or IFN-γ were measured implicating T H1 overactivity in the pathogenesis of uremic pruritus.\textsuperscript{162} Calcineurin is protein phosphatase 2B which induces IL-2 expression in T-cells by activation of the transcription factor NFATc. The calcineurin-inhibitor cyclosporine A down-regulates IL-2 synthesis and rapidly improved pruritus in patients with atopic dermatitis\textsuperscript{163} and therapy-resistant Sézary-syndrome.\textsuperscript{164} In rats, a discrete population of cutaneous C-polymodal nociceptors was activated by IL-2.\textsuperscript{165} These cutaneous C-fibres also responded to bradykinin and histamine, and bradykinin increased their responsiveness to IL-2.\textsuperscript{166} Transgenic mice over-expressing IL-4 in the epidermis manifested with a pruritic skin disorder that fulfilled the clinical diagnostic criteria established for atopic dermatitis in patients.\textsuperscript{167} In patients with pruritus due to HIV infection levels of T H2-cytokine levels including IL-4, IL-5 and IL-10 were increased.\textsuperscript{143} Furthermore, IL-6 levels were reported to be increased in haemodialysis patients with pruritus compared to those patients without pruritus indicating a role of inflammation in the pathogenesis of uremic itch.\textsuperscript{168} In the dermis of prurigo nodularis lesions clusters of nerve-like fibres with enhanced IL-6-like immunoreactivity were observed which co-localized with CGRP expression.\textsuperscript{169}

The level of the T H2-cytokine interleukin-13 has been reported to be increased in serum of atopic dermatitis patients.\textsuperscript{170} IL-13 signals through a receptor dimer consisting of IL-13Rα1 and IL-4Rα which activates members of the JAK/STAT pathway resulting in phosphorylation of STAT-6 as well as induction of PI3-kinase and MAP kinase signalling cascades.\textsuperscript{171} A functional link of IL-13 to pruritus has been made by Zheng et al.\textsuperscript{8} using an inducible IL-13 transgenic mouse model in which IL-13 was overexpressed exclusively in keratinocytes. These mice developed a chronic inflammatory phenotype similar to atopic dermatitis characterized by xerosis, pruritic eczematous lesions and increased scratching behaviour associated with increased synthesis of IL-4 and IL-13 by CD4\textsuperscript{+} T-cells.\textsuperscript{8}

More recently, a novel interleukin, IL-31, was described which is produced by T H2-cells as well as mast cells and signals through a receptor complex composed of IL-31 receptor A and oncostatin M subunits.\textsuperscript{9,172} Upon binding of IL-31 the receptor activates members of the JAK family of tyrosine kinases which cause activation of the transcription factor STAT-1 and -5, Erk-1/2 as well as induction of PI3-kinase and MAP kinase
signalling cascades.\textsuperscript{173,174} Mice overexpressing IL-31 developed severe pruritus, alopecia and skin inflammation.\textsuperscript{9} In Nc/Nga mice which spontaneously develop atopic dermatitis-like skin lesions mRNA levels of IL-31 correlated with the number of scratch bouts\textsuperscript{175} and intraperitoneally applied monoclonal anti-IL-31 antibodies ameliorated scratching behaviour without improving skin lesions and dermatitis.\textsuperscript{176} In humans, IL-31 levels were also increased in serum of patients with chronic urticaria and atopic dermatitis and even correlated with disease severity in atopic dermatitis patients.\textsuperscript{177,178} IL-31 expression was increased in prurigo nodularis and lesional skin of atopic dermatitis patients.\textsuperscript{179} IL-31 expressing T-cells could recently be identified in lesional skin of atopic dermatitis patients which were in part co-expressing IL-13 and to a lesser extent IL-22.\textsuperscript{180} Notably, a substantial part of these IL-31 expressing T-cells did not co-express any typical cytokine indicating a novel entity of yet undefined T-cells.\textsuperscript{180} Furthermore, treatment of skin with staphylococcal superantigens rapidly induced IL-31 expression in atopic individuals.\textsuperscript{179} The antimicrobial peptides human \(\beta\)-defensin and cathelicidin have been shown to induce IL-31 expression in human mast cells.\textsuperscript{172} Mast cells generated from CD34\(^+\) mononuclear cells from polycythemia vera patients released higher amounts of interleukin-31 resulting in increased IL-31 plasma levels in these patients.\textsuperscript{181} Next to keratinocytes, IL-31 receptors have also been described on DRG neurons\textsuperscript{179,182} indicating that IL-31 could directly activate sensory nerve fibers. These results underline that IL-31 and its receptors represent a novel target for anti-pruritic therapy.

**Opioids**

In 1975, Hughes and colleagues identified two endogenous peptides, Leu- and Met-enkephaline, with a potent opiate-like activity.\textsuperscript{183} Later it was shown that these enkephalines and their receptors were present in central and peripheral neuronal tissue.\textsuperscript{184} Administration of these peptides and other opioid agonists relieves pain, but causes itching as a side-effect – the mode of action, however, differs between peripheral and central application.\textsuperscript{56} Intradermal injection of morphine and other opioid agonists caused local itching which was associated by a wheal and flare reaction.\textsuperscript{185} Of note, \(H_1\)-antihistamines but not the \(\mu\)-opioid-antagonist naloxone effectively attenuated local itch sensation,
indicating that pruritus due to intradermal injection of opioids is mediated by histamine release.\textsuperscript{185,186} Interestingly, low doses of opioids which did not produce itch sensation when injected alone, potentiated histamine-induced pruritus when co-injected with histamine (as similarly described for prostaglandins).\textsuperscript{187} On the other hand itch sensation was neither attenuated by local pretreatment with compound 48/80 to deplete histamine from mast cells nor by oral pretreatment with indomethacin to inhibit prostaglandin synthesis indicating that the potentiating effect of histamine-induced itching was not caused by histamine release or prostaglandin formation.\textsuperscript{187} Similarly, naloxone did not diminish pruritus in this study suggesting that the peripheral pruritic effects of opioids are mediated via others than μ-opioid receptors.

Generalized pruritus after oral, subcutaneous or intravenous application of opioid agonists is observed in around 1% of patients, whereas up to 90% report itching after epidural or intrathecal injection.\textsuperscript{22,188} Itching upon epidural injection may be confined to the segmental area of anti-nociception but often also spreads rostrally and affects in particular the nose and the face of the patients.\textsuperscript{21} As wheal or flare reactions are not observed in these areas and antihistamines do not relieve pruritus, this form of itching is histamine-independent. In contrast, naloxone effectively inhibited itching from spinal opioids.\textsuperscript{21} Similar observations were made in various animal models.\textsuperscript{189-192} Thus, central opioid receptors are capable of mediating itch sensation. This effect was previously explained by the occlusion hypothesis which assumed that itch and pain signals are selectively mediated via nociceptive and pruriceptive spinal neurons\textsuperscript{193} and that itch signalling is under inhibitory control of pain neurons which fails upon inactivation of pain signalling, e.g. by morphine.\textsuperscript{21,193,194} This theory was supported by the analysis of mice lacking the vesicular glutamate transporter 2 (VGLUT2) in subsets of nociceptive DRG neurons disconnecting them from their spinal targets that resulted in markedly increased scratch behaviour accompanied by an attenuated responsiveness to thermal pain.\textsuperscript{195} However, an elegant study\textsuperscript{6} could recently clearly show that long lasting morphine-induced itching occurred independently of the morphine-induced analgesia and was mediated via the μ-opioid receptor (MOR1), whereas short-lived morphine-induced scratching was mediated specifically via the isoform D of this receptor (MOR1D) which is located on distinct sets of neurons.\textsuperscript{6} Intriguingly, MOR1D forms a heterodimer with the gastrin-releasing peptide
Neuronal circuits in pruritus of systemic disorders

receptor (GRPR) and morphine-induced scratching was mediated by activation of GRPR. As morphine-induced analgesia remains unaffected in GRPR deficient mice, GRPR seems to characterize a purely itch-related spinal interneuron. These observations strongly favour a modified labelled line hypothesis, in which itch and pain sensation are mediated via distinct sets not only of presynaptic but also of postsynaptic neurons, and GRPR-expressing interneurons are likely to represent the itch-specific relays in the spinal cord. In contrast to μ-opioid agonists which cause scratching, κ-opioid agonists such as nalfurafine have been shown to inhibit scratching in animal models and patients suffering from uremic pruritus. By which mode of action and via which receptor(s) κ-opioid agonists suppress pruritus and cause analgesia at the same time remains to be elucidated.

Interestingly, the bovine adrenal medulla peptide BAM(8-22) is derived from proenkephalin A was proven to induce scratch responses after intradermal injection in mice via activation of MrgC11 (see below). Thus, peripherally mediated itch sensation by certain opioids may be mediated by others than opioid receptors.

Endocannabinoids

Endocannabinoids which are derived from the essential fatty acid arachidonic acid comprise a group of substances that modulate pain and itch sensation by binding to cannabinoid (CB) receptors. CB1-receptors are found in the CNS, whereas CB2-receptors are mainly expressed in peripheral tissues. Interestingly, CB1- and TRPV1-receptors showed a marked co-localisation in primary afferent C-fibres. In addition, both receptors are expressed in cells of the skin including keratinocytes and mast cells. Cannabinoid agonists strongly attenuated histamine-induced scratching behaviour in mice and could reduce itch severity in humans. In rats, activation of keratinocytes located around nerve endings in the epidermis via a CB2 receptor agonist caused secretion of β-endorphin. The released β-endorphins bound to μ-opioid receptors of afferent nerve fibres thereby inhibiting nociception. Moreover, endocannabinoids like anandamide – which in higher concentrations may also act as an endovanilloid – have been shown to
activate and eventually desensitize TRPV1 demonstrating the complex role of cannabinoids in the modulation of pruritus and pain. Thus, endocannabinoids do not only participate in pain sensation, but may also modulate pruritoception.

**Neurotrophins**

Neurotrophins such as nerve growth factor (NGF), brain-derived neurotropic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) belong to a class of growth factors which modulate survival, differentiation, and maintenance of nerve cells. In the skin, these proteins are synthesized and released by mast cells, eosinophils, keratinocytes and fibroblasts. Of note, expression of NGF was markedly increased in lesional skin of patients with atopic dermatitis or psoriasis which caused sensitization as well as sprouting of sensory nerve fibres by activation of p75 and tyrosine kinase neurotrophin receptors (TrkA receptors). Several studies showed that NGF can upregulate the expression of sensory neuropeptides such as substance P or CGRP, may sensitize the transient receptor potential vanilloid-1 (TRPV1) and potently cause degranulation of mast cells thereby leading to acute itch sensation. In addition, substance P and CGRP increased synthesis of NGF in human cultured keratinocytes, indicating that a positive forward loop could be responsible for sensitization of sensory neurons. Furthermore, serum levels of NGF and BDNF were increased in patients with atopic dermatitis and correlated with disease severity. In mouse models of atopic dermatitis NGF and glial cell line-derived neurotrophic factor (GDNF), another neurotropic factor, were upregulated which could contribute to scratching behaviour. Thus, neurotrophins play an important role in the sensitization of afferent neurons in various skin disorders and it would be interesting to investigate their role in systemic diseases associated with pruritus.
Phospholipids

- **Lysophosphatidic acid**

Lysophosphatidic acid (LPA) is a potent lipid mediator exerting its effects via at least six distinct LPA-receptors. Intradermally injected LPA induced a scratch response in mice in a dose-dependent manner. Screening sera of cholestatic patients for neuronal activation revealed LPA as major activator (see also hepatobiliary disorders).

- **Sphingosinephosphorylcholine**

Intradermally injected sphingosinephosphorylcholine (SPC) caused scratching behaviour in mice. Interestingly, SPC concentrations were increased in lesional skin of NC/Nga mice. SPC-induced pruritus was partly explained by synthesis and secretion of LTB4 from keratinocytes as scratching behaviour was diminished by a 5-lipoxygenase inhibitor and a LTB4-antagonist. On the other hand SPC increased cytosolic free calcium concentrations in DRG neurons indicating that SPC is capable of directly activating sensory neurons.

- **Platelet-activated factor**

Intradermal injection of platelet-activated factor (PAF) caused vasodilatation and increased vascular permeability, producing a weal and flare response with accompanying pruritus. Thus, PAF-induced pruritus is thought to be caused by liberation of histamine from mast cells. PAF may also play a role in pruritus associated with allergic conjunctivitis.

Others

- **Acetylcholine**

Acetylcholine (ACh) is a major neurotransmitter of the peripheral and central nervous system and exerts its effects via muscarinergic and nicotinergic acetylcholine receptors. Whereas intracutaneous injection of high concentrations of acetylcholine caused pain sensation in healthy volunteers, a pure itching sensation was reported after injection in lesional skin of patients with atopic dermatitis. Blockade of H1-receptors did neither
diminish acetylcholine-induced itch severity nor reduce erythema in patients with atopic dermatitis, but reduced pain sensation and erythema in healthy controls.\textsuperscript{231} In line, patients with atopic dermatitis exhibit a reduced sensitivity to histamine compared to healthy controls and pruritus does not respond to H\textsubscript{1}-antihistamines.\textsuperscript{232} Hence, in patients with atopic dermatitis pruritus can be elicited by a cholinergic, histamine-independent mechanism. In mice, acetylcholine was shown to cause scratch responses mainly via the activation of muscarinergic acetylcholine receptor M3,\textsuperscript{233} which is a member of G\alpha\textsubscript{q} GPCRs and thus signals via activation of phospholipase C. M3 receptors are expressed on keratinocytes but not dermal and epidermal nerve endings – in contrast to inhibitory M2 receptors.\textsuperscript{234} In addition, it should be noted that excitatory and CGRP-releasing effects are evoked by nicotinergic AChRs.\textsuperscript{234}

- **Bradykinin**

Bradykinin is a nonapeptide which is liberated from kininogens through hydrolytic cleavage by kallikreins. Kallikreins, the enzymes forming bradykinin, are serine proteases which have for decades been known to cause severe itching\textsuperscript{122} without inducing a significant flare or wheal reaction\textsuperscript{235} (see also proteases). Bradykinin is known as an inflammatory pain mediator by activating bradykinin B\textsubscript{2} receptors on sensory neurons, but may under certain circumstances also cause pruritus.\textsuperscript{235-237} Bradykinin and kallidin (also entitled lysylbradykinin as it has the same amino acid sequence of bradykinin with an additional lysine), a decapeptide which is also generated by the action of kallikreins on kininogens, are potent histamine releasers from mast cells, and itch sensations caused are inhibited by antihistamines.\textsuperscript{75,235} Furthermore, bradykinin sensitized sensory afferents for various chemical stimuli and augmented their response to subsequent histamine application.\textsuperscript{238,239} This effect may be caused by the bradykinin-mediated release of substance P, CGRP and PGE\textsubscript{2} after application to the skin.\textsuperscript{98} Furthermore, bradykinin – similar to acetylcholine\textsuperscript{230,231} – has been reported to induce intense itching in lesional skin of atopic dermatitis patients which was not diminished by antihistamines.\textsuperscript{240} In inflamed skin induced by complete Freund’s adjuvant bradykinin caused robust scratching behaviour which was mediated via B\textsubscript{1} receptors which are induced upon inflammation.\textsuperscript{241} Thus, the algogen bradykinin may under certain circumstances cause histamine-independent pruritus.
Interestingly, bradykinin has been shown by several groups to activate both TRPV1 and TRPA1 channels which have been associated with pain and itch signalling. This activation appears to be mediated by phospholipase C.242

**Mrg receptors**

The antimalaria drug chloroquine induces pruritus in humans, particular in those persons with dark skin such as black Africans. In experimental itch research chloroquine has long been used for the induction of histamine-independent pruritus; however, the molecular mechanisms of itch induction remained unresolved until recently. Liu and colleagues elegantly proved that chloroquine elicits scratching behaviour in mice by binding to a GPCR (Gαq) called MrgA3 (Mas-related gene GPCR subtype A3).4 Its human correlate is MrgX1. Conversely, the peptide BAM8-22 elicited itch through MrgC11. The Mrg receptors constitute a subfamily of GPCRs that are largely orphan receptors but recently have been associated to sensory functions. In contrast to the extensive sequence diversity exhibited by the MrgA, MrgB, and MrgC subfamilies in mice (n>50), the related complement in humans is composed of only a few MrgX genes (n~9), while that in rats counts one each of the MrgA, MrgC, and MrgD genes and ten MrgB genes. Although the human MrgX genes have no direct orthologs in rodents, human MrgX1 has been suggested to share some features of expression, binding profile and structural homology with rat...
MrgC and mouse MrgC11. Expression of many of these receptors under normal conditions seems to be confined to neurons and mast cells.\textsuperscript{243}

Induction of a scratch response via MrgA3 does neither activate phospholipase C $\beta_3$ (PLC$\beta_3$)\textsuperscript{10} nor requires TRPV1\textsuperscript{11} as shown in knock-out animals, making it distinct from the histamine-1-receptor pathway (see fig. 5 for schematic representation). Still, MrgA3\textsuperscript{+} neurons also respond to histamine.\textsuperscript{4} In fact, a different TRP effector is required for chloroquine-induced scratching behaviour, namely TRPA1.\textsuperscript{12} Scratch responses after intradermal injection of chloroquine were almost abolished in TRPA1 deficient mice.\textsuperscript{12} Activation of MrgA3 was shown to act through liberation of G$\beta\gamma$, a signalling molecule which modulates several ion channels by direct binding.\textsuperscript{12} Whether G$\beta\gamma$ directly opens TRPA1 channels or acts via an indirect mechanism remains to be elucidated. Furthermore, PIRT, a phosphoinositide-interacting regulator of transient receptor potential channels, is required for chloroquine-induced scratch responses.\textsuperscript{33} Thus, coupling of GPCR to TRP channels seems to represent a common event in various itch (and pain) signalling pathways. Han and colleagues genetically modified and ablated MrgA3\textsuperscript{+} DRG neurons (representing around 5\% of sensory neurons) resulting in attenuation of scratching behaviour to multiple pruritogens while pain behaviour was unaltered.\textsuperscript{13} Using an elegant approach this group expressed TRPV1 solely in MrgA3\textsuperscript{+} neurons in TRPV1\textsuperscript{-/-} mice. Activation of these MrgA3\textsuperscript{+} sensory neurons by the algogen and TRPV1-agonist capsaicin elicited only hindpaw scratching but no pain-related forepaw wiping.\textsuperscript{13} These findings suggest that MrgA3 defines a specific subpopulation of DRG neurons responsible for itch signalling underlying the labelled line theory initially suggested by Johannes Müller in 1826.\textsuperscript{244} Still, Han and co-workers could not test cowhage in these mice, although in vitro all MrgA3\textsuperscript{+} neurons were activated by cowhage. Species differences complicate the picture as cowhage does excite all polymodal nociceptors in humans, but only a subset of histamine-sensitive ones in mice.\textsuperscript{245} Thus, MrgA3 is likely to represent a suitable candidate for novel anti-pruritic treatment strategies for several but not all forms of pruritus.
HAEMATOLOGIC DISORDERS

Polycythemia vera

Polycythemia vera (PV) is a rare haematological disorder characterized by Janus kinase 2 (JAK2) mutations resulting in a erythroid-weighted trilineage myeloproliferation. The most common JAK2 mutation results in a substitution of valine to phenylalanine at codon 617 (JAK2V617F) and renders the kinase constitutively active and makes hematopoetic cells more sensitive to growth factors. Notably, pruritus appears to correlate with homozygosity for the gain-of-function mutation JAK2V617F. Two independent studies showed that patients with cellular homozygosity suffered more often from pruritus than patients with cellular heterozygosity. The mutation has not been detected in either control subjects or germline tissue, confirming that the allele is not a common polymorphism in the general population. Generalized pruritus is reported by 30–65% of patients suffering from polycythemia vera and typically described as aquagenic pruritus. Besides polycythemia vera, aquagenic pruritus may also occur in other myeloproliferative disorders such as essential thrombocytosis, primary myelofibrosis and the hypereosinophilic syndrome (see below) or as an isolated symptom in healthy people. Aquagenic pruritus in patients with polycythemia vera is reported to have a typical prickling, stinging or burning character which particularly occurs directly after exposure to water and lasts for 10–120 minutes (average 30–45 min).

The pathophysiology of polycythemia vera-associated pruritus remains largely unknown, but several hypotheses mainly based on clinical observations have been made. The sudden onset and limited duration of itch sensation with characteristic occurrence after (but not during) exposure to water indicates that a sudden decrease in skin temperature triggers the initiation of pruritus. Cooling down the skin by evaporating humidity from the skin might cause a release of adenosine diphosphate from red blood cells and catecholamines from adrenergic vasoconstrictor nerves resulting in blood vessel contraction and activation of platelets. Platelets may then release pruritogenic factors such as
prostaglandin E₂ (PGE₂) and serotonin. Although both substances are weak pruritogens upon single intradermal application, an intradermally injected mixture of PGE₂ and serotonin caused strong pruritus in healthy controls.⁵⁶ The beneficial effect of pitozifen,²⁵⁴ a serotonin and weak histamine antagonist, was explained by reducing the pruritogenic effect of released serotonin from platelets.⁵⁶ The selective serotonin re-uptake inhibitor paroxetine and fluoxetine also strongly reduced polycythemia vera-associated pruritus.²⁵⁰,²⁵⁵ As these drugs mainly act centrally, they might influence the central itch signalling pathway rather than acting directly on peripheral nerve endings which do not re-uptake 5-HT but express 5-HT receptors. However, platelets cannot synthesize 5-HT but take it up from enterochromaffine cells during passage through the gut. Degranulation of platelets is known to be selectively inhibited by the cyclooxygenase inhibitor acetylsalicylic acid,²⁵⁶ which could explain the beneficial anti-pruritic effect of this drug in polycythemia vera patients.⁵⁷,¹⁴¹ It is intriguing to speculate whether platelet-derived lysophosphatidic acid,²⁵⁷ another potential pruritogen (see above), is involved in polycythemia vera-associated pruritus. After a hot bath lysophosphatidic acid may be released from endothelial cells to cause contraction of smooth muscle cells thereby restoring vascular tone and it could additionally activate platelets to release further lysophosphatidic acid and other pruritogens.²⁵⁸,²⁵⁹ Mast cells may be another potential source of pruritogens in polycythemia vera patients. Various pruritogenic mediators can be secreted by mast cells among which are histamine, tryptase, interleukins and prostaglandins. Although serum levels of histamine were raised in polycythemia vera,²⁴⁹,²⁶⁰ which could be explained by an increased number of basophile granulocytes in those patients,²⁶¹ they do not develop urticaria and antihistamines are not or only partially effective in these patients.¹⁴¹,²⁵⁰,²⁶² Tryptase levels as a specific marker for mast cell degranulation were not found to be increased one hour after showering,²⁶³ however a subtle or local release of mast cell granula may not be detected in peripheral blood. Jackson et al. found an increased number of skin mast cells in patients suffering from pruritus compared to those without pruritus and healthy controls.¹⁴¹ These mast cells were located throughout the dermis, but concentrated in particular around small blood vessels.¹⁴¹ A more recent study could histologically show that mast cells degranulated after exposure to warm water in polycythemia vera patients but not in healthy controls, although their number was not increased compared to controls.²⁶⁴
Interestingly, the number of cutaneous mononuclear cells and eosinophils was highly elevated after water exposure.\textsuperscript{264} An older publication reported about a similar increase in mononuclear cells in the dermis and epidermis in a female polycythemia vera patient who underwent biopsies before and after warm water challenge.\textsuperscript{265} Mast cells generated from CD34\textsuperscript{+} mononuclear cells of polycythemia vera patients released higher amounts of pruritogenic substances such as histamine, leukotrienes, and interleukin-31 and had an increased migratory behaviour compared to mast cells of healthy controls.\textsuperscript{181} Noteworthy, a greater number of mast cells could be generated from polycythemia vera patients suffering from pruritus compared to patients without pruritus. These mast cells were less prone to apoptosis and released more pruritogenic cytokines such as IL-31, resulting in increased IL-31 plasma levels in these patients.\textsuperscript{181} Finally, the same group demonstrated that all mast cells of polycythemia vera patients had the malignant gene mutation JAK2 617V>F. This could explain the beneficial anti-pruritic effect of newly developed JAK inhibitors such as erlotinib,\textsuperscript{266} ruxolitinib (INCB018424)\textsuperscript{267-269} or TG101348.\textsuperscript{270} One could also argue that the anti-pruritic effect of these substances may be due to their efficient decrease in disease burden. A strong improvement of pruritus is often reached by an effective treatment of the underlying disease – indicated by correction of haematological parameters – as shown by various trials using busulphan,\textsuperscript{141} hydroxyurea,\textsuperscript{271} interferon-\textalpha or its pegylated form (reviewed in \textsuperscript{272}) or recently by a phase II trial using the histone-deacetylase inhibitor givinostat.\textsuperscript{273} Beside IL-31, other interleukins have been suggested to cause pruritus of PV. A recent study could, however, not find any correlation between IL-10, IL-22 or IL-23 and the presence of pruritus.\textsuperscript{274}

Phlebotomy is performed to treat polycythemia vera, but might cause iron deficiency anaemia in these patients. As iron deficiency is also associated with pruritus (see also section iron deficiency of this review), iron supplementation has been given to polycythemia vera patients with iron deficiency anaemia and relieved pruritus in some of these patients.\textsuperscript{275-277} However, when iron treatment had to be stopped because of unacceptably high haemoglobin concentrations, pruritus recurred.\textsuperscript{277} Thus, iron supplementation should be restricted to those iron deficiency anaemia patients with otherwise untreatable pruritus.
**Essential thrombocytosis**

Essential thrombocytosis (ET) is a clonal disorder arising from a pluripotent hematopoietic stem cell characterized by a platelet-weighted trilineage myeloproliferation.\(^{278}\) Similar to polycythemia vera the gain-of-function mutation JAK2V617F, which renders hematopoietic cells more sensitive to growth factors, has been described in approximately 30% of patients with essential thrombocytosis.\(^{246,279}\) In an international internet-based survey of 1179 patients suffering from different myeloproliferative diseases, pruritus was reported in 40% of essential thrombocytosis patients.\(^{252}\) Interestingly, in contrast to polycythemia vera, pruritus was reported in a similar frequency by essential thrombocytosis patients being homozygotic for the JAKV617F mutation compared to heterzygotic patients.\(^{247}\) Pruritus in patients with essential thrombocytosis is described as an aquagenic pruritus similar to polycythemia vera-associated pruritus indicating that the pathogenesis of this agonizing sensation may be similar in both disorders (see polycythemia vera). This is further strengthened by the fact that in first clinical trials the JAK1 and JAK2 inhibitor ruxolitinib (INCB018424)\(^{267-269}\) also remarkably improved pruritus in essential thrombocytosis patients suggesting that certain pruritogenic cytokines signalling via the JAK-STAT-pathway might be involved in the pathogenesis of pruritus.

**Primary myelofibrosis**

Primary myelofibrosis (PMF) is characterized by fibrotic changes of the bone marrow and an extramedullary hematopoiesis. It represents the third large entity of myeloproliferative diseases which is commonly associated with pruritus. Approximately every second patient with primary myelofibrosis reported about itching in a large survey of more than 450 patients.\(^{252}\) Myelofibrosis might also occur in the spent phase of polycythemia vera and essential thrombocytosis possibly due to bone marrow stem cell toxic drugs such as hydroxyurea. Similar to polycythemia vera and essential thrombocytosis, the valine-to-phenylalanine change mutation in the JAK2 gene (JAK2V617F) is found in 50% of primary myelofibrosis patients. Selective JAK inhibitors erlotinib \(^{266}\), ruxolitinib (INCB018424)\(^{267-269}\) or TG101348\(^{270}\) have been reported to consistently improve pruritus.
and other constitutional symptoms such as fatigue in these patients. Similarly, everolimus, an inhibitor of the rapamycin target mTOR \(^{280}\) and the histone-deacetylase inhibitor givinostat (ITF2357) \(^{273}\) also significantly reduced pruritus in PMF patients, however the underlying mechanism remains elusive. As itch sensation in all myeloproliferative disorders is commonly reported as aquagenic pruritus similar underlying pathogenic causes (see polycythemia vera) may be presumed.

**Hypereosinophilic syndrome**

The hypereosinophilic syndrome (HES) consists of a very rare and heterogeneous group of syndromes defined as persistently increased eosinophil count greater than \(1.5 \times 10^9/L\) for more than six months without any recognizable cause and eosinophil-associated organ damage. \(^{281,282}\) Recent advances in the understanding of the underlying pathogenesis have established that hypereosinophilia may – among other causes – be due to (i) an increased interleukin-5 synthesis by a T cell clone (lymphocytic HES), (ii) a chromosomal rearrangement resulting in fusion of the two genes, FIP1-like 1 (FIP1L1) and platelet derived growth factor alpha (PDGFRA) on chromosome 4q12 in hematopoetic stem cells, which results in clonal hypereosinophilia or (iii) a myeloproliferative disorders (m-HES) of which the causes have not been elucidated yet. \(^{281,282}\) Beside IL-5 other cytokines such as IL-3 or granulocyte-macrophage stimulating colony factor (GM-CSF) have been discussed as causing pruritogenicity. Itch sensation in HES patients may present as aquagenic pruritus, \(^{283}\) but could also be caused by pruritic papules and eczema-like nodules. \(^{284}\) Efficient treatment of the underlying disease resolves the constitutive symptoms such as pruritus which can be achieved by either corticosteroids \(^{285}\) or the monoclonal anti-IL-5 antibody mepolizumab. \(^{286}\)

**Myelodysplastic syndrome**

The myelodysplastic syndromes comprise a group of diverse hematological conditions characterized by ineffective or dysplastic production of blood cells associated with an
increased risk for acute leukaemia.\textsuperscript{287,288} Pruritus has occasionally been reported in these patients and, similar to the myeloproliferative syndromes, has many features of aquatic pruritus.\textsuperscript{289-292} Psoralen phototherapy has in casuistics been described to effectively improve pruritus in these patients, however the underlying beneficial mechanisms remain unclear.\textsuperscript{289,291}

\textit{Mastocytosis}

The term mastocytosis refers to a group of rare disorders being characterized by too many mast cells and their CD34\textsuperscript{+} progenitors.\textsuperscript{293} Mastocytosis is subdivided into cutaneous forms which are confined to the skin and preferentially seen in children and systemic mastocytosis in which mast cells infiltrate extracutaneous organs with or without skin involvement.\textsuperscript{294} Mast cells express a specific tyrosine kinase receptor called c-kit (CD117), which is activated by stem cell factor (SCF).\textsuperscript{295} Mutations in c-kit have been linked to the pathogenesis of mastocytosis and the most common mutation which is seen in more than 90\% of systemic mastocytosis patients consists of the substitution of valine for aspartate in codon 816 (D816V).\textsuperscript{296,297} This mutation causes ligand-independent auto-phosphorylation of c-kit and induces constitutive activation of the Stat5-PI3K-Akt signalling cascade.\textsuperscript{298} Pruritus is one of the most common symptoms reported by mastocytosis patients and arises from the release of various mediators by mast cells.\textsuperscript{299,300} Itch sensation can be triggered in those patients by a variety of stimuli among which are physical factors such as mechanical and thermal stimuli including pressure or massage of the skin, extremes in temperature or sudden changes in temperature, exercise, emotional stress, infections, surgery or certain medications.\textsuperscript{299-302} Histamine might play an essential role in the pathogenesis of pruritus in mastocytosis as H\textsubscript{1}-histamine receptor blockers\textsuperscript{303} and the mast cell stabilizer sodium chromoglycate\textsuperscript{304} have been effective in treating pruritus. However, one report questioned the role of histamine as main pruritogenic agent as blocking histamine synthesis in two mastocytosis patients did not improve pruritus.\textsuperscript{305} Treatment with the irreversible histidine-decarboxylase inhibitor α-fluoromethylhistidine resulted in decreased levels of plasma histamine and urinary metabolites but solely diarrhea improved and seemed thus to be the only histamine-related symptom.\textsuperscript{305} Those patients who are unresponsive to histamine
Neuronal circuits in pruritus of systemic disorders

receptor blockers may be treated by anti-leukotriene agents such as montelukast to improve pruritus.306

**Hodgkin’s lymphoma**

Hodgkin’s lymphoma, also known as Hodgkin’s disease, is a malignancy of lymphocytes characterized by multi-nucleated CD30+/CD15+ Reed-Sternberg and Hodgkin cells.307 The prevalence of pruritus in Hodgkin’s disease has been reported in 15–30% of patients.308-310 Pruritus tends to be located in the area drained by the lymphatic vessels of affected lymph nodes, but may also be generalized particularly in patients with the nodular sclerosis type of Hodgkin’s disease.311 It may present with ichthyosiform skin changes on the extremities or as eczema lesion, but is often only associated with secondary skin lesions due to intense scratching.312

The following mechanisms have been suggested in the pathogenesis of pruritus in Hodgkin lymphoma: (i) release of bradykinin and cytokines as immune response to the malignant lymphoid cells,311,313 (ii) histamine release from eosinophils observed in the pleotropic infiltrates of Hodgkin lymphomas,311,313 and (iii) high serum levels of IgE with specific cutaneous IgE deposits seen in 10–20% of patients with lymphoma.311,313 Furthermore, it is intriguing to speculate on the role of autotaxin in relation to pruritus and tumour progression in patients with Hodgkin’s disease. Recently, Epstein-Barr virus-infected Hodgkin lymphoma cells have been shown to highly express the lysophospholipase D, ATX. This enzyme is responsible for the formation of LPA, which has been shown to induce pruritus in a dose-dependent manner in mice.223,224 These cells might thus release high levels of ATX, leading to high local concentrations of LPA that may not only promote tumour growth314 but also activate the pruritoceptive C fibres (see below). The role of these factors in pathogenesis of itch and tumour progression in lymphoma patients warrants further investigations.

Specific therapeutic options for pruritus in Hodgkin lymphoma patients are lacking, but effective tumour treatment is regularly associated with attenuation or relief of itching.
Corticosteroids are commonly reported in textbooks to be effective in these patients, however studies supporting this observation are lacking. As this drug class is part of the regular treatment regimen it is unclear whether its effectiveness is due to tumor eradication or a real anti-pruritic effect. Interestingly, H<sub>2</sub>-antihistamines such as cimetidine were reported as effective symptomatic anti-pruritic drugs.\textsuperscript{315}

**Other haematological malignancies**

The prevalence of chronic pruritus in other haematological diseases has never been studied in detail and numbers are based on small studies and case series. It is estimated that up to 10% of patients with Non-Hodgkin’s lymphoma and up to 5% of leukemic patients suffer from itching.\textsuperscript{316} Pruritus is more frequently observed in lymphocytic than in myeloid leukemia and more common in chronic than in acute disease.\textsuperscript{313} Severe itching is common in CD4<sup>+</sup> mycosis fungoides and its leukemic form, the Sézary’s syndrome, which are Non-Hodgkin T-cell lymphoma with primary manifestation in the skin. These tumours are characterized by a constitutive STAT3 expression,\textsuperscript{317} a key transcription factor for the Th2 cytokine production, resulting in increased levels of Th2 cytokines such as IL-4, IL-5, and IL-10.\textsuperscript{318,319} Beside these cytokines, pruritus in patients with the Sézary syndrome may be caused by increased levels of substance P as aprepitant, a selective neurokinin-1-receptor antagonist, largely diminished itch severity in three patients.\textsuperscript{93}

**Iron deficiency**

The association of iron deficiency with generalized pruritus is known for decades,\textsuperscript{320} which is often believed to be a rare phenomenon. However, a prospective French study revealed that 5% of patients with generalized pruritus in the absence of a dermatological disorder suffered from iron deficiency.\textsuperscript{321} A large Finish cohort of several ten thousand adults revealed that 13.6% of men and 7.4% of women with anaemia due to iron deficiency experienced pruritus.\textsuperscript{322} As iron deficiency can be associated with a malignant disorder,\textsuperscript{323} itching may represent a paraneoplastic symptom.\textsuperscript{312} However, restoring serum iron and
ferritin to normal levels by oral iron supplementation normally resolves pruritus in anemic patients within days to weeks. In male patients with generalized pruritus and low serum iron levels, screening for myeloproliferative disorders is indicated.
Chapter 2

METABOLIC AND ENDOCRINE DISORDERS

Hepatobiliary disorders

Pruritus is a frequently observed symptom accompanying many hepatobiliary disorders, particularly those with cholestatic features. This form of itching is designated cholestatic pruritus as bile secretion and/or flow is impaired in these disorders. In these disorders, cholestasis may be caused by a pure hepatocellular secretory failure as seen in intrahepatic cholestasis of pregnancy (ICP), benign recurrent intrahepatic cholestasis, progressive familial intrahepatic cholestasis, toxin- or drug-induced cholestasis, and chronic viral hepatitis B and C infections. Intrahepatic bile duct damage and secondary hepatocyte secretory failure is causing cholestasis in primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and paediatric cholestatic syndromes such as the Alagille syndrome. Finally, cholestasis due to obstruction of the intrahepatic or extrahepatic bile duct system is observed in cholestasis caused by gallstones, PSC, cholangiocellular carcinoma, obstructive tumours of the pancreatic head or enlarged lymph nodes located in the hilar region, bile duct adenomas, or biliary atresia. Interestingly, the prevalence of pruritus varies considerably between these hepatobiliary disorders. Pruritus is the defining symptom of women suffering from ICP and is experienced by up to 80% of patients with PBC and PSC at any time during the course of their disease. In contrast, pruritus is less frequently reported by patients with obstructive cholestasis, chronic hepatitis C infections, and rarely associated with chronic hepatitis B patients, non-alcoholic fatty liver disease, alcoholic or non-alcoholic steatohepatitis even when cholestasis is present.

Pruritus of cholestasis is characterized by a circadian rhythm with patients reporting the highest intensity in the evening and early at night, a predilection site at the limbs and in particular at the palms and soles, and the phenomenon that scratching barely alleviates itch sensations. Furthermore, female cholestatic patients commonly report pruritus worsening during progesterone phase of the menstrual cycle, in late pregnancy, and during hormone replacement therapy. In multivariate analysis, serum alkaline phosphatase and the Mayo risk score were found to be independent indicators for the
Neuronal circuits in pruritus of systemic disorders

occurrence of pruritus in 335 PBC patients. The Mayo risk score is derived from an equation containing clinical variables including patient age, serum total bilirubin, albumin, prothrombin time, and the presence/absence of edema or ascites. Various substances among which are histamine, bile salts, endogenous opioids and progesterone metabolites have been proposed but with limited evidence as pruritogens in the past. Recently, we could identify the potent neuronal activator lysophosphatidic acid as potential pruritogen in cholestasis. Still the molecular mechanisms of cholestatic pruritus remain to be unravelled. The current understanding of the potential pruritogen(s) is mirrored by the effects of therapeutic approaches aiming i) to remove the pruritogen(s) from the enterohepatic cycle by non-absorbable, anion exchange resins such as cholestyramine, colestipol, and colesevelam in mild pruritus or interventions such as external biliary diversion, nasobiliary and transcutaneous drainage in desperate cases; ii) to remove the potential pruritogen(s) from the systemic circulation by invasive procedures such as anion absorption, plasmapheresis or extracorporeal albumin dialysis; iii) to alter the metabolism of the presumed pruritogen(s) in the liver and/or the gut by biotransformation enzyme inducers such as rifampicin or iv) to modify central itch and/or pain signalling by influencing the endogenous opioidergic and serotonergic system via \( \mu \)-opioid-antagonists and selective serotonin re-uptake inhibitors, respectively. The presence of direct or indirect pruritogens in the enterohepatic circulation is highlighted by the dramatic effects of interruption of this circulation either by nasobiliary drainage, which usually relieves itch within 24 hours. 

Potential factors involved in cholestatic pruritus

- **Histamine**

Elevated levels of histamine have been found in plasma of patients with chronic cholestatic liver disorders and in cholestatic animal models. Hydrophobic bile salts, in particular deoxycholate, chenodeoxycholate, and their conjugates, are capable to release histamine from mast cells, albeit at concentrations that are much higher than those generally observed in cholestatic patients. Cholestatic patients do not present with typical histamine-induced skin alterations such as erythema, urticaria and flares and antihistamines are
commonly ineffective in this form of pruritus. Furthermore, we could show that tryptase levels which represent a specific marker for activation of mast cells, were similar in cholestatic patients with and without pruritus. Thus, histamine is unlikely to represent a direct pruritogen in cholestatic pruritus.

- **Serotonin**

Serotonin (= 5-HT = 5-hydroxytryptamine), which is synthesized from the amino acid tryptophan, has been reported to excite nociceptive nerve fibers. As serotonergic receptors modulate the transmission of opioid pain-inhibitory signals in the brain, serotonin might also play a role in itch signalling. Furthermore, serotonin induces itch in humans when it is injected intradermally or applied via iontophoresis. Enhanced scratching activity was also observed in mice after injection of serotonin.

Several clinical studies investigated the antipruritic effect of the 5-HT3-receptor antagonist, ondansetron, in cholestatic patients with conflicting results. Interestingly, the serotonin reuptake inhibitor sertraline was shown to moderately improve pruritus in cholestatic patients. It was suggested that this obviously paradoxical effect is due to the dichotomous effects of serotonin on central versus peripheral nervous system. Thus, it appears that serotonin may modulate sensation of pruritus in cholestasis, but does not represent a key pruritogen in cholestasis.

- **Bile salts**

For decades bile salts have been held responsible for cholestatic itch as bile salts accumulate during cholestasis, caused pruritus in healthy volunteers upon intradermal injection, and aggravated pruritus of cholestatic patients after oral supplementation. This hypothesis was strengthened by the observation that binding of bile salts inside the intestinal lumen by anion exchange resins such as cholestyramine, colestipol, and colesvelam ameliorated pruritus and removal of bile from the body by
Neuronal circuits in pruritus of systemic disorders

ileal exclusion surgery,\textsuperscript{368,369} transcutaneous\textsuperscript{370-374} and nasobiliary drainage\textsuperscript{343,344,375} rapidly alleviated long-lasting intractable pruritus. A recent study suggested that cholestatic pruritus is mediated by the bile salt GPCR TGR5 which was detected in sensory neurons of mouse dorsal root ganglia.\textsuperscript{376} Indeed, intradermal injection of high concentrations of the bile salts deoxycholate and lithocholate induced scratching behaviour which was attenuated in TGR5\textsuperscript{-/-} mice.\textsuperscript{376} TGR5 transgenic mice exhibited spontaneous scratching behaviour which was augmented by interdermal injection of bile salts. Bile salt-induced neuronal activation and scratching behaviour was dependent on the presence of TRPA1.\textsuperscript{377} However, the applied concentrations of these hydrophobic bile salts were far beyond the pathophysiological levels observed during cholestasis. Other, agonists of TGR5 such as neurosteroids might however be capable of activating this receptor leading to itch sensation. This remains to be investigated.

Several studies could not prove any correlation between severity of pruritus and concentrations of any naturally occurring bile salt in the circulation, urine or skin.\textsuperscript{338,349,378-380} Furthermore, frequency and intensity of cholestatic itch does not correlate with the severity of cholestasis.\textsuperscript{381} This is underlined by the fact that pruritus may be the initial presenting symptom in early stages of PBC patients when bile salts are low, but is often lost in terminal liver failure when bile salts reach their highest concentrations.\textsuperscript{328} Patients with obstructive cholestasis may have highly increased bile salts levels but never experience itching.\textsuperscript{382} In contrast, women with ICP have marginally increased serum bile salts but do by definition suffer from pruritus.\textsuperscript{329} Itching can ameliorate spontaneously or may even vanish despite ongoing cholestasis and unaltered raised levels of bile salts.\textsuperscript{381,382} Cholestyramine improved pruritus not only in cholestasis but also in polycythemia rubra vera, a hematological disorder which is not associated with elevated bile salts.\textsuperscript{383} Colesevelam efficiently decreased serum bile salt concentrations by approximately 50\% in a randomized, multicenter study but improvement of pruritus was similar to that of placebo.\textsuperscript{384} The enzyme inducers phenobarbital and rifampicin effectively improved pruritus without changing the levels of serum bile salts.\textsuperscript{385,386} Anecdotal treatments included androgens such as methandrostenolone which relieved pruritus but worsened cholestasis and raised serum bile salts.\textsuperscript{387,388} Finally, bile salt concentrations did not correlate with itch
intensity in PBC patients undergoing nasobiliary drainage.\textsuperscript{344} In summary, bile salts and their metabolites play at best an indirect role in the pathogenesis of pruritus of cholestasis.

- **Steroids**

Female steroid hormones and their metabolites have been implied in the pathogenesis of cholestatic pruritus\textsuperscript{328} based on several observations. Intradermal application of pruritogens caused stronger scratching behaviour in female compared to male mice.\textsuperscript{389} Similarly, female cholestatic patients reported pruritus to be more intense and more frequent compared to men.\textsuperscript{390} During pregnancy concentrations of steroids and steroid metabolites continuously rise\textsuperscript{391} reaching the highest levels during the last trimester when intrahepatic cholestasis of pregnancy typically occurs.\textsuperscript{329,392,393} One study found a slight correlation between reduction of urinary levels of disulphated progesterone metabolites and improvement of pruritus in ICP patients treated with ursodeoxycholic acid, whereas neither bile salt metabolites nor other steroid metabolites showed a similar correlation.\textsuperscript{392,394} In addition, Abu Hayyeh et al.\textsuperscript{395} recently reported a significant increase in the serum epiallopregnanolone sulphate concentration in ICP women as compared to normal pregnant women. It may be noted that reduced dehydroepiandrosterone sulfate (DHEAS) levels significantly correlated with fatigue severity in patients with primary biliary cirrhosis,\textsuperscript{396} whereas the plasma concentrations of the precursor molecules DHEA and pregnenolone remained within the levels of controls, further strengthening the potential role of neurosteroids as mediators of central nervous symptoms associated with cholestasis. As steroid hormones and their metabolites are capable of influencing many ionotropic receptors such as transient receptor potential vanilloid 1 (TRPV1),\textsuperscript{397} GABA-A,\textsuperscript{398} glycine,\textsuperscript{399} glutamate,\textsuperscript{400-403} and serotonin receptors,\textsuperscript{404} they might modulate neuronal excitability and thereby influence pruritoception and/or nociception in cholestatic patients.
• **Endogenous opioids**

Since the report of Bernstein and Swift that the pruritus of cholestasis in a woman with PBC disappeared after an intravenous infusion of the μ-opioid antagonist naloxone, endogenous opioids such as Met- and Leu-enkephalins have been discussed as pruritogens in cholestasis. The central administration of opiate agonist drugs can induce itching in human beings and scratching activity in animals. Levels of endogenous opioids were raised in bile duct resected rats as well as in plasma of cholestatic PBC patients. Furthermore, spinaly administered plasma extracts from pruritic cholestatic patients induced facial scratching behaviour in monkeys, which was abolished by co-administering naloxone. In bile duct resected rats opioid receptors were downregulated in certain parts of the brain which might represent consequence of increased exposure of opioid receptors to endogenous opioids. The liver may be the source of increased opioid levels during cholestasis as mRNA expression of preproenkephalin was increased in liver of bile duct-resected rats and Met-enkephalin immunohistochemical staining was raised in the periportal areas and proliferating bile ductules of cholestatic rat livers. The importance of the altered endogenous opioid system in pruritus of cholestatic patients is underlined by the moderate anti-pruritic effect of μ-opioid receptor antagonists such as naloxone, naltrexone, and nalmefene.

Nonetheless, several facts argue against a direct role of endogenous opioids as pruritogens in cholestasis. A correlation between itch intensity and endogenous opioid concentrations has never been shown. Similar opioid levels were measured in PBC patients with and without pruritus showing a relation with the stage of disease in PBC patients rather than with the presence of pruritus. In PBC patients Met-enkephalin concentrations were significantly raised in histological stage 3 and 4, whereas pruritus is commonly reported in early stages of this disease and rather improves in end-stage liver disease. Women with ICP who by definition suffer from pruritus, had a similar μ-opioid activity compared to gestation-matched pregnant controls. The antinoceptive and pruritopepticeffect seen in cholestasis was explained by an increased central opioidergic tone. However, a recent study proved that the antinoceptive effect of endogenous opioids in a cholestatic mouse model was caused by local effects at the level of
peripheral sensory nerve endings, but not a central mechanism. There is evidence to suggest that endogenous opioids may partly contribute to pruritus in cholestasis, but are not likely to represent the causative pruritogens.

**Treatment of cholestatic pruritus**

As the pathogenesis of cholestatic pruritus is still poorly understood, medical and interventional treatment options are limited. Therapeutic efforts should include an adequate therapy of the underlying hepatobiliary disease which may result in relief of pruritus. Pruritus due to extrahepatic biliary obstruction is effectively treated by endoscopic biliary stenting, transcutaneous or nasobiliary drainage, or surgical biliodigestive anastomoses. In contrast, pruritus due to intrahepatic cholestasis may represent an enormous therapeutic challenge in some affected patients. Table 1 summarizes validated and experimental treatment options for pruritus in cholestatic patients.

<table>
<thead>
<tr>
<th>Drug/therapy</th>
<th>Final dose</th>
<th>Recommendation/evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Efficacy proven in controlled trials</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>10–15 mg/kg/d (PO)³</td>
<td>I A – II C⁴</td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>4–16 g/d (PO)⁴</td>
<td>I B – IIb C⁵</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>300–600 mg/d (PO)</td>
<td>I A</td>
</tr>
<tr>
<td>Natamycin</td>
<td>10 mg/kg/d (PO)³</td>
<td>I A</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>25–50 mg/d (PO)</td>
<td>I A</td>
</tr>
<tr>
<td>Naloxone</td>
<td>0.2 μg/kg/min (IV)</td>
<td>I B</td>
</tr>
<tr>
<td>Serotonin reuptake inhibitors (e.g. sertraline)</td>
<td>75–100 mg/d (PO)</td>
<td>Ila B</td>
</tr>
<tr>
<td><strong>Contradictory efficacy observed in controlled trials</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ondansetron</td>
<td>4–24 mg/d (PO)</td>
<td>II A</td>
</tr>
<tr>
<td></td>
<td>4–8 mg/d (IV)</td>
<td></td>
</tr>
<tr>
<td><strong>Efficacy shown in case series or case reports</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propofol</td>
<td>10–15 mg (IV bolus)</td>
<td>Ila B</td>
</tr>
<tr>
<td></td>
<td>1 mg/kg/h (IV)</td>
<td></td>
</tr>
<tr>
<td>Lidocaine</td>
<td>100 mg/d (IV)</td>
<td>Ila B</td>
</tr>
<tr>
<td>Dronabinol</td>
<td>15 mg/d (PO)</td>
<td>Iib C</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>1–2 mg/d (intranasal)</td>
<td>Iib C</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>2–5 mg/kg/d (PO)</td>
<td>Iib B</td>
</tr>
<tr>
<td>Phototherapy (UVA, UVB)</td>
<td></td>
<td>Iib C</td>
</tr>
<tr>
<td>Bright light therapy reflected towards the eyes</td>
<td>10 000 lux 60–120 min/d</td>
<td>Iib C</td>
</tr>
<tr>
<td>Plasmapheresis, extracorporal albumin dialysis (e.g. MARS), plasma separation, anion absorption, nasobiliary drainage, biliary diversion</td>
<td></td>
<td>Ila C</td>
</tr>
<tr>
<td>Liver transplantation</td>
<td></td>
<td>I C</td>
</tr>
</tbody>
</table>

Table 1: Therapeutic strategies for pruritus in cholestasis.
Ursodeoxycholic acid

Ursodeoxycholic acid (UDCA) constitutes up to 3% of the human bile acid pool. Upon oral administration, however, UDCA forms up to 50% of bile acids and thereby renders the bile acid pool more hydrophilic.\textsuperscript{426,427} In primary biliary cirrhosis (PBC), the most common chronic cholestatic liver disease, UDCA represents the only approved medical treatment. It improves serum liver tests including cholestatic markers, delays progression to fibrosis and cirrhosis, diminishes the rate of complications, normalizes life expectancy in early stage disease, and may prolong transplant-free survival in a large cohort of patients with stage I – IV.\textsuperscript{428} Due to its anti-cholestatic effect, UDCA is also administered in other cholestatic disorders like primary sclerosing cholangitis, intrahepatic cholestasis of pregnancy, cystic fibrosis-associated liver disease, and pediatric cholestatic syndromes. Several mechanisms and sites of action of UDCA have been unravelled: \textit{i}) UDCA improves the impaired hepatobiliary secretion by stimulating posttranslational synthesis, targeting and apical insertion of key hepatocellular transporters into their target membrane and also enhances cholangiocyte secretion, \textit{ii}) detoxifies bile, and \textit{iii}) exerts anti-apoptotic effects both in hepatocytes and cholangiocytes.\textsuperscript{327} UDCA is well-tolerated and causes diarrhea only exceptionally.\textsuperscript{429} The effect of UDCA on pruritus has never been specifically addressed by therapeutic trials testing antipruritogenic strategies. In randomized, placebo-controlled trials for treatment of PBC or PSC, UDCA has not been convincingly shown to alleviate pruritus in patients affected by this symptom.\textsuperscript{341,430} However, small trials reported antipruritic effects of UDCA in children with cholestatic disorders.\textsuperscript{431-433} Furthermore, UDCA is a safe and effective therapy in women with intrahepatic cholestasis of pregnancy (ICP) in whom it improved intensity of pruritus, serum liver tests, time of delivery, and birth weight of the neonates.\textsuperscript{394,434,435}

Anion exchange resins

Anion exchange resins are non-absorbable, alkaline macromolecules binding anions and amphipathic substances including bile salts in the gut lumen and thus preventing their reuptake in the terminal ileum. Cholestyramine and colestipol represent two resins which
have been extensively used to treat cholestatic pruritus. Up to 80% of patients responded within two weeks completely or partially to this drug.\textsuperscript{367,436-439} A starting dose of 4 g cholestyramine once or twice a day is recommended, which can be extended to 4 x 4 g. As pruritogens presumably accumulate in the gallbladder overnight, accumulation of anion exchange resins is recommended as a 4 g dose one hour before and after breakfast. Anion exchange resins interfere with the absorption of several drugs like UDCA, digoxin, warfarin, propranolol, and oral contraceptive hormones as well as fat-soluble vitamins. Thus, anion exchange resins should be taken at least four hours prior to any other medication.\textsuperscript{440} Adverse effects include abdominal discomfort, bloating, diarrhea, hypertriglyceridemia, and rarely bleeding after long-term use. Colesevelam, a novel bile acid sequestrant being initially used to treat hypercholesterolemia, has superior binding affinities for bile acids and other amphiphilic substances than cholestyramine and colestipol and was reported to have no major gastrointestinal side-effects.\textsuperscript{441} Its effect on pruritus needs to be evaluated in controlled trials.

**Rifampicin**

The antibiotic rifampicin is used for the treatment of tuberculosis since decades. This semi-synthetic compound derived from Amycolatopsis rifamycinica induces phase I and II enzymes and key membrane transporters in the liver by activation of the nuclear steroid and xenobiotic pregnane X receptor (PXR).\textsuperscript{442} It induces the expression of phase I biotransformation enzymes like CYP3A4, CYP2D6, and other members of the microsomal cytochrome P\textsubscript{450} system, phase II biotransformation enzymes like bilirubin-conjugating enzyme UGT1A1 or sulfotransferase SULT2A1, and phase III export pumps like canalicular conjugate export pump MRP2.\textsuperscript{443,444} Thus, rifampicin accelerates detoxification and excretion of numerous compounds like bilirubin, bile acids, steroids, and drugs. A possible explanation for the antipruritic effect of rifampicin might be an enhanced metabolism and/or increased secretion of direct or indirect pruritogens. Furthermore, its antimicrobial effect on the intestinal flora might alter intestinal metabolism of pruritogens. However, its antipruritic effect cannot be only due to increased CYP3A4 activity as
phenobarbital led to a similar induction of CYP3A4 but was inferior regarding the effect on pruritus.\textsuperscript{445}

Rifampicin at doses of 300-600 mg/d\textsuperscript{386,445,446} and 10 mg/kg/d,\textsuperscript{447} respectively, was reported to improve pruritus in cholestasis. Rifampicin was effective also in children with chronic cholestasis.\textsuperscript{448,449} Recent meta-analyses of prospective randomized, controlled trials revealed that rifampicin is an effective and safe short-term treatment of pruritus,\textsuperscript{450,451} whereas hepatotoxicity has been observed in up to 13\% of patients after 3 months by some,\textsuperscript{447} but not all cohorts during long-term follow-up up to 72 months.\textsuperscript{386,446,449} Thus, serum transaminase levels should be monitored at regular intervals when rifampicin is prescribed.\textsuperscript{452} Patients should be informed that rifampicin changes the colour of urine and tears to orange-red, a benign but sometimes frightening side effect.

**Opioid antagonists**

Almost 30 years ago, Bernstein et al. reported on amelioration of cholestatic pruritus in a patient with PBC by the opioid antagonist naloxone.\textsuperscript{405} Several clinical trials have proven the positive effect of opioid antagonists on pruritus in patients with hepatobiliary diseases.\textsuperscript{451} Naloxone (given as an intravenous bolus of 0.4 mg followed by continuous infusion of 0.2 \(\mu\)g/kg/min),\textsuperscript{416,417} nalmefene (60-120 mg/d; orally),\textsuperscript{410,420} and naltrexone (25-50 mg/d; orally)\textsuperscript{418,419,421,422} significantly reduced itch and/or scratching behaviour. Nalmefene is not any more available in most countries. Parenterally administered naloxone should be reserved for emergency treatment. Naltrexone was proven to be more effective than placebo in reducing pruritus as well as in improving fatigue and depression.\textsuperscript{419,421} Opioid antagonists are well tolerated during long-term treatment, but severe opiate withdrawal-like reactions during the first days of treatment possibly due to an enhanced opioidergic tone in cholestatic patients have been reported.\textsuperscript{410} Therefore, opioid antagonists should be started at very low doses. Alternatively, treatment could be either initiated with intravenous naloxone at sub-therapeutical doses (e.g. 0.002 \(\mu\)g/kg/min), then gradually increased before switching to oral naltrexone\textsuperscript{453} or co-administered with clonidine (100 mg t.i.d.) which can be tapered within one week.\textsuperscript{410} Pruritus may recur during long-term opioid
antagonist therapy possibly due to drug-induced upregulation of $\mu$-opioid receptors. This breakthrough phenomenon may be prevented by interrupting treatment for two days of the week, e.g. on Saturday and Sundays.\end{footnote}

The $\kappa$-opioid receptor agonist nalfurafine improved pruritus in patients with uremic pruritus$^{198}$ and the $\mu$-opioid receptor antagonist and $\kappa$-opioid receptor agonist butorphanol (1 mg intranasally) alleviated pruritus in a patient with chronic hepatitis C infection.\end{footnote} Thus, $\kappa$-opioid receptor agonists may become new treatment options for cholestatic pruritus in the near future.

**Serotonin antagonists (5-HT$_3$-antagonists) and selective reuptake-inhibitors (SSRI)**

Initial studies using subjective methodology reported that intravenous administration of the 5-HT$_3$-antagonist, ondansetron, markedly reduced pruritus within hours in patients suffering from cholestatic liver diseases$^{355,356}$ and intrahepatic cholestasis of pregnancy.\end{footnote} Controversial results were reported for oral administration of 5-HT$_3$-antagonists. Only a minor benefit could be demonstrated in a study using visual analog scale for evaluation of pruritus intensity,\end{footnote} but these results were not confirmed when intensity of pruritus was analyzed by objective methodology using a scratching activity monitoring system (SAMS).\end{footnote} Thus, the effectiveness of 5-HT$_3$-antagonists for the treatment of pruritus in cholestasis remains questionable. In otherwise intractable pruritus, experimental use of intravenously administered 5-HT$_3$-antagonists may be justified.

The serotonin-reuptake inhibitors (SSRI) sertraline$^{67,357}$ and paroxetine$^{69}$ have been reported to improve pruritus in cholestasis and advanced cancer stages. It remains unclear whether the apparently paradoxical effect of these antidepressants is due to dichotomous effects of serotonin on the central versus the peripheral nerve system,\end{footnote} to downregulation of excitatory 5-HT$_3$-receptors, or to a modification of central opioid receptors.\end{footnote}
Cannabinoids

Cannabis was listed in the United States Pharmacopeia from 1850 until 1942. However, due to its widespread illegal use, its licensed prescription is nowadays controversial. Various cannabinoids from the cannabis plant are ligands of cannabinoid receptors. Dronabinol is a sesame oil preparation of the semi-synthetic analog of Δ⁹-tetrahydrocannabinol (THC), a psychoactive compound of cannabis sativa (= marijuana). In three patients with intractable cholestatic pruritus, 5 mg of dronabinol every eight hours temporarily relieved itch and improved sleep and depression. Interestingly, chronic pruritus of different origin was attenuated after topical application of the cannabinoid receptor agonist N-palmitoylethanolamine. Cannabinoids might increase the threshold for the perception of pruritus via stimulation of cannabinoid/opioid receptor interactions on nerve fibers. However, these preliminary observations require further investigations in randomized, placebo-controlled clinical trials.

Others

Enzyme Inducers

Phenobarbital is a ligand of the nuclear constitutive androgen receptor (CAR) and induces isoenzymes of the cytochrome P₄₅₀ family similar to rifampicin. In a randomized, controlled, cross-over study phenobarbital attenuated pruritus in cholestasis, but was less effective than rifampicin. Other hepatic enzyme inducers like flumecinol and the androgen stanozolol have been reported to attenuate cholestatic pruritus in small case series. The use of stanozolol is limited by the fact that it worsened cholestasis.

Anesthetics

Propofol at subhypnotic doses (15 mg i.v.) relieved cholestatic pruritus in ten patients with various liver diseases in a prospective, cross-over, placebo-controlled trial. Propofol
presumably inhibits afferent C-fibers in the dorsal horn of the spinal cord rather than being antipruritic via sedation.\textsuperscript{21}

Lidocaine (100 mg i.v.) alleviated pruritus and fatigue in a small cohort of PBC patients when compared to placebo.\textsuperscript{461}

\textit{S-adenosyl-L-methionine (SAMe)}

Several clinical trails tested the efficacy of SAMe in comparison to placebo or UDCA in women with ICP.\textsuperscript{462-464} Based on their outcome, SAMe cannot be recommended for treatment of pruritus in ICP.

\textit{Phototherapy}

Phototherapy with ultraviolet light (UV-A, UV-B) on the skin\textsuperscript{465,466} was reported to alleviate pruritus in cholestatic patients. Chemical modifications of pruritogens in the skin or altered skin sensitivity to pruritogens have been discussed as potential mechanisms of action. Randomized, controlled trials are lacking.

Bright light therapy towards the eyes (one hour, twice daily) improved pruritus in single patients,\textsuperscript{467} but has been associated with episodes of mania among other side effects and should therefore be only applied under controlled conditions.

\textit{Extracorporeal elimination of pruritogens}

A beneficial effect of therapeutic procedures such as plasmapheresis,\textsuperscript{468} molecular adsorbent recirculating system (MARS) therapy,\textsuperscript{469,470} plasma separation and anion absorption,\textsuperscript{471} partial external diversion of bile,\textsuperscript{369} ileal diversion in children,\textsuperscript{472} and nasobiliary drainage in children\textsuperscript{343} and adults\textsuperscript{344} with otherwise uncontrollable pruritus has been reported in case series. Their temporary success supports the view that putative
pruritogens in cholestasis accumulate in plasma and undergo an enterohepatic circulation. However, none of the studies were placebo-controlled and the techniques are invasive, very elaborate, and too expensive for routine use. Thus, they should only be considered for otherwise intractable pruritus in desperate patients.

Liver transplantation

A successful liver transplantation cures the underlying disease and pruritus is relieved quickly. In patients in whom severe pruritus is refractory to all above mentioned treatments, this symptom may become an indication for liver transplantation even in the absence of liver failure.326,473-475

A step-by-step recommendation for treatment of pruritus in cholestasis (Figure 6)

Ursodeoxycholic acid (UDCA, 10-15 mg/kg/d) is regarded as effective first line treatment of pruritus in intrahepatic cholestasis of pregnancy and exerts anticholestatic effects in various other cholestatic disorders. Anion exchange resins like cholestyramine (4 g before and after breakfast; maximum: 16 g/d) are a first therapeutic step in pruritus of all other forms of intrahepatic cholestasis and extrahepatic forms in which bile flow cannot be restored by invasive procedures. If ineffective, cholestyramine should be stopped after two weeks and 150 mg rifampicin should be applied b.i.d., which may be increased to a maximum of 600 mg/d. If no response to therapy is achieved within two weeks, rifampicin should be discontinued. Naltrexone is recommended as third-line therapy. Withdrawal-like reactions can be avoided by start with low doses of 12.5 mg/d (or intravenous naloxone infusions, see above). The antidepressant sertraline 75 mg/d can be administered as fourth-line therapy. Pruritus will improve in most patients in response to these treatment strategies. In patients who do not adequately respond to standard treatment, alternative approaches could be considered as outlined in Table 1. Co-administration of several drugs at the same time is not recommended due to the risk of drug-drug interactions.
Figure 6: Step-by-step recommendation for treatment of pruritus in cholestasis. PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis; ICP: intrahepatic cholestasis of pregnancy; UDCA: ursodeoxycholic acid.
Chronic renal disorders

Chronic kidney disease (CKD) carries substantial morbidity and mortality and can contribute to the dysfunction of many organ systems. The cutaneous manifestations of renal failure and dialysis are wide-ranging but most commonly include dyspigmentation, pruritus, ecchymosis, perforating dermatosis, xerosis, acne, bullous disorders, calciphylaxis, pallor, and hair and nail abnormalities. Pruritus often occurs in patients with chronic kidney disease (15–49%) and is present in a majority (50–90%) of patients with end-stage renal disease on dialysis. The prevalence of uremic pruritus in patients receiving peritoneal dialysis versus haemodialysis is comparable. It occurs independent of age, sex, race, aetiology of renal failure and has been described to occur at all times throughout the day, worsening at night, and affecting large non-dermatomal based and symmetric skin areas. Uremic pruritus can be severely debilitating, negatively impacting sleep quality, overall quality of life, and mortality, but nonetheless is often under-recognized throughout the nephrology community.

The pathogenesis of uremic pruritus remains largely unknown. Many hypotheses have been generated in attempt to explain this poorly understood but highly present and persistent symptom. Some of these include metabolic by-product accumulation, immune system dysfunction, endogenous opioidergic system dysregulation, cutaneous barrier dysfunction, alterations in afferent nerve function, increased number of dermal mast cells and their products, increased formation of insoluble calcium phosphate, hyperparathyroidism, and hypervitaminosis A.

Blood levels of urea nitrogen and creatinine has been utilized as markers for accumulation of yet unidentified metabolic products. Recently, there have been significant advances in haemodialysis technology and membrane filtration has become more sophisticated; it is now possible to filter a wider range of molecules with larger molecular weight such as $\beta_2$-microglobulin and others that are implicated in contributing to uremic pruritus. Data comparing the effects of these newer synthetic filtration methods on pruritus reduction have been mixed. However, a recent randomized, prospective, double-blind study was performed to compare the efficacy of high permeability haemodialysis against conventional haemodialysis on uremic pruritus. A decrease in parathyroid hormone
and β2-microglobulin and pruritus was demonstrated. To that end, another study showed a modest reduction in pruritus in patients who underwent haemodialysis daily versus three times weekly (34% vs. 13%).

A complex interplay between the immune system and the nervous system is increasingly recognized in the pathophysiology of inflammation. An imbalance of the highly regulated interactions of the skin, immune, and nervous systems in the skin contribute to inflammation and itch sensation. Chronic renal failure is thought to initiate and maintain a state of chronic low-grade inflammation that contributes to pruritus. Tachykinins such as substance P can activate transcription factors producing proinflammatory cytokines (e.g. IL-2) and when injected intradermally, substance P causes pruritus. There have been several studies investigating non-specific markers of inflammation in patients on haemodialysis. In pruritic patients requiring haemodialysis (approximately one-third of patients in the study), elevated c-reactive protein (CRP) levels were found as compared to those on haemodialysis without pruritus. The authors also noted that other inflammatory markers including α1-acid-glycoprotein, β2-microglobulin, ferritin, and albumin were equivalent in both groups. In another study, patients on chronic haemodialysis with moderate or severe pruritus as measured using a visual analog scale (VAS) also had elevated CRP levels with equivalent levels for TNF-α. These patients had a higher all-cause mortality rate than those without pruritus, further supporting a role for chronic inflammation bridging uremic pruritus and poor outcome. Serum CRP and IL-6 levels were elevated in plasma of patients with uremic pruritus compared to patients without pruritus, although an older study found no differences between these cytokines. Many immunosuppressive therapies including ultraviolet light, γ-linolenic acid, thalidomide, and tacrolimus improve pruritus thus providing complementary support for inflammation in the genesis of uremic pruritus. Interestingly, patients with glomerulopathies have a statistically significant lower prevalence of pruritus compared to patients with renal failure due to other etiologies. Those patients are often treated with immunosuppressive therapies which may serve to globally suppress the low-grade inflammation associated with classical renal failure and the development of pruritus. Finally, post-renal transplant patients rarely experience pruritus during the period in which
they are taking systemic immunosuppressive medications, even with recurrent renal failure.\textsuperscript{479,485}

The dermis of patients with chronic kidney disease maintains an increased number of mast cells and this has been thought to contribute to the prevalence of pruritus in CKD.\textsuperscript{493} Mast cells are known to release many (putative) pruritogens including histamine, cytokines (e.g. TNF-\textit{\alpha} and IL-2), and proteases (e.g. tryptase and chymase). Although many studies have attempted to demonstrate a correlation between serum histamine levels and uremic pruritus, no consistent relationship has been observed.\textsuperscript{38,504} In addition, oral antihistamines are not effective in treating uremic pruritus. In parallel, there is a clear elevation of serum tryptase levels in patients on haemodialysis as compared to the general population.\textsuperscript{488,505} In one study, the mast cell stabilizer cromalyn sodium was used to treat uremic pruritus over an 8-week period; the treatment group experienced a significant decrease in mean pruritus as measured on a VAS.\textsuperscript{488} Notably, with treatment and symptomatic improvement there was only a slight decrease in serum tryptase values. These observations leave the possibility that other mast cell mediators besides histamine or tryptase contribute to pruritus or that the local dermal concentrations of these molecules and not the serum levels are determining stimulation of small afferent C-fiber neurons involved in itch sensation.

Xerosis is a common dermatologic condition and its incidence is increased in patients with renal failure. Xerosis is even more increased (up to 85\% of patients) in those receiving haemodialysis.\textsuperscript{506} It is not often seen in acute renal failure and does not correlate with plasma urea levels; however, it often resolves after renal transplantation. A distinct causal relationship between uremic xerosis and uremic pruritus has been suggested in the literature, but is not undisputed.\textsuperscript{506-509} Clinical observations showed that many patients with marked xerosis do not suffer from itch. Still, pruritus is often reported to be improved by moisturizing and rehydrating the skin.\textsuperscript{506} The skin of patients with uremic xerosis displays low hydration of the stratum corneum and atrophy of the sebaceous and secretory sweat glands with decreased sweating, but normal barrier function.\textsuperscript{506,509-511} Based upon this and other histopathologic differences, uremic xerosis is an independent feature of dialysis patients and can be distinguished from other causes of dry skin. Lipid imbalance has been
postulated to contribute to the xerosis of renal failure. Finally, stratum corneum pH was shown to be elevated in haemodialysis patients. These skin alterations may contribute to the intensity chronic kidney disease associated pruritus, however, do not represent the cause of induction of itch sensation.

Special attention has been focused on neuropathic changes, nerve proliferations of the pruritus-mediating cells, and central nervous alterations. In patients with chronic kidney disease a correlation between paraesthesia and presence of uremic pruritus has been described. Accumulation of uremic toxins may result in damage of sensory nerve fibres resulting in uremic neuropathy and central sensitization to itch. Histamine iontophoresis in patients undergoing haemodialysis caused smaller flare reactions but increased itch sensation compared to healthy volunteers confirming an altered neurophysiological response. Furthermore, the anticonvulsant gabapentin, which is widely used as pain-modulating drug in patients with neuropathic pain, has been shown to exert strong anti-pruritic effect in chronic kidney disease-associated pruritus. Similar beneficial results have recently been reported for pregabalin. Thus, neuropathic changes seem at least in part be responsible for the agonizing pruritus in chronic kidney disease.

Iron deficiency, iron deficiency anaemia and anaemia of chronic disease have also been hypothesized to contribute to pruritus of renal failure. In states of iron deficiency, there is an up-regulation of interleukin-6 and induction of hepcidin and thus can be regarded as a state of chronic inflammation. It has been proposed that zinc deficiency may contribute to uremic pruritus via activation of the histamine pathway, but a recent case-control study did not find any correlation between zinc levels, parathyroid hormone levels and pruritus.

Hyperparathyroidism has also been implicated in contributing to uremic pruritus, although there have not been many reliable studies showing a positive association. In fact, recent data suggest that there is no correlation between parathyroid hormone levels and intensity of pruritus in uraemia. Nonetheless, in two small independent studies almost 35 years apart, parathyroidectomy has been curative for the symptoms of itch in most cases. The symptomatic relief occurred within 1–4 weeks post-operatively, and correlated with normalization of the parathyroid hormone levels. Chronic renal failure is often
accompanied by hyperphosphataemia, hypocalcaemia, and decreased calcitrol thus causing secondary hyperparathyroidism. In addition, only when the calcium-phosphorus product is exceedingly high there is an increase in uremic pruritus. The consequences of this are largely unknown, with the distinct possibility of an increased calcium-phosphorus product leading to increased dermal calcium deposition, which may cause modulation of neuronal signalling in C fibers.

Opioid peptides have long been implicated as mediators of pruritus in chronic kidney disease. Pruritus caused by cutaneous injection of compounds including histamine, substance P, serotonin, and acetylcholine has been shown to be suppressed by naloxone and naltrexone. However, no correlation could ever be established between opioid levels and severity of pruritus or serum levels of β-endorphins before and after dialysis. Still, clinical studies have shown that suppression of the activity of opioid responsive neurons by μ-opioid receptor antagonists results in attenuation of itch sensation in cholestasis, uraemia, and generalized pruritus. A placebo-controlled, double-blind crossover study of uremic patients with persistent, treatment-resistant pruritus naltrexone did, however, not improve itching. Recently, an imbalance between μ- and κ-opioid tone has been hypothesized to contribute to uraemia associated and generalized pruritus. κ-opioid agonists alone have demonstrated an inhibition of substance P and histamine induced scratching in mice, and to reduce pruritus associated with uraemia in humans, albeit to a mild extent. Modulation of the endogenous opioidergic tone in the nervous system may be caused by substances accumulating during chronic kidney disease, however, these substances seem not to represent the causal pruritogens of uremic pruritus.

The pathogenesis of uremic pruritus remains obscure. Various substances have been discussed as potential pruritogens in chronic renal diseases; however, a causal relation could never be established. Over the past few decades, numerous treatment approaches have been employed in attempt to alleviate renal pruritus. Like other sub-classes of pruritus, there is no magic bullet that can be used to treat this problem and a treatment ladder is often applied.
**Endocrinological disorders**

**Diabetes mellitus**

Two independent large cohorts of several hundred diabetic patients indicated that only up to 3% suffered from generalized pruritus. Increased glucose levels cause non-enzymatic glycation of various structures in the body among which are neurons. Neuropathy is commonly observed in diabetic patients mainly as distal symmetric polyneuropathy associated with abnormal, nociceptive, burning or prickling sensations and less often with pruritus. In a diabetic rat model it was recently shown that dorsal root ganglia had a reduced expression of cannabinoid (CB) 1-receptors. Diabetic neuropathy might therefore be explained by the loss of the neuroprotective effect of cannabinoids.

**Thyroid disorders**

Pruritus has been reported in up to 11% of patients with hyperthyreosis and in particular of those with thyrotoxicosis due to long-lasting, untreated Graves’ Disease. The pathophysiological mechanisms leading to itch sensation in hyperthyreosis are unclear. It was suggested that excessive amounts of thyroid hormones may activate kinins due to increased tissue metabolism or that the threshold of itch sensation could be lowered due to warmth and vasodilatation. Pruritus may also be triggered by chronic urticaria which are caused by the underlying thyroid immunity. Notably, up to 12% of patients with chronic urticaria suffered from autoimmune thyroid disorder. It is likely that pruritus is caused by xerosis cutis as most patients respond to emollients. Pruritus in dry skin may be caused by a complex cross-talk between dysregulated keratinocytes, pruritogenic cytokines such as thymic stromal lymphopoietin (TSLP) or TNF-α and epidermal sensory nerve endings.
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Parathyroid disorders

Pruritus can be a symptom of primary hyperparathyroidism; however, pruritus has mainly been studied in the context of secondary hyperparathyroidism in uremic patients (see also renal disorders). Secondary hyperparathyroidism is induced in these patients by reduced calcium levels due to (i) diminished renal conversion of vitamin D into its active form and (ii) increased circulating levels of phosphate leading to the formation of insoluble calcium phosphate in the body. Subtotal parathyroidectomy in uremic patients was associated with partial or complete relief of pruritus. In addition, increased levels of parathyroid hormone (PTH) were found in uremic patients with pruritus compared to those without. However, PTH seems not to be the causative pruritogen as (i) immunohistochemical investigations of skin biopsies from uremic patients against PTH were negative, (ii) pruritus was not seen in all patients with secondary hyperparathyroidism, (iii) increased PTH levels were not always associated with pruritus, and (iv) levels of PTH did not correlate with itch severity. Thus, clear evidence for PTH as a direct role in the pathogenesis of pruritus in hyperparathyroidism and uraemia is lacking.

Hemochromatosis

Hereditary haemochromatosis is a common autosomal-recessive metabolic disorder characterized by iron accumulation in various organs of the body. Generalized pruritus is a very rare complication in these patients as underlined by only a few cases reported in the literature. The pathogenesis of pruritus related to haemochromatosis is totally unclear, but might be caused by direct stimulation of pruritoceptive fibres by iron ions or iron deposits in the skin which activate mast cells to release pruritogenic substances.

Anorexia nervosa

Almost 20 years ago it has been suggested that starvation-associated pruritus should be considered as a clinical symptom of eating disorders. Indeed, pruritus has been reported
in 16–58% of women suffering from anorexia nervosa.\textsuperscript{551,552} In these patients a correlation between body-mass-index and itch intensity could be established and pruritus strongly improved upon weight restoration.\textsuperscript{552} Xerosis cutis has been accused as pathogenetic mechanism in starvation-associated pruritus which is observed in almost 60% of women with anorexia nervosa.\textsuperscript{551}

\textit{Lactose intolerance}

Beside anorexia nervosa, pruritus may also be observed in various malabsorption syndromes e.g. chronic inflammatory bowel diseases, celiac disease (see below) and lactose intolerance. Of note, in patients with aquagenic pruritus of unknown origin a subgroup of 25% (which had no haematological disorder or any other laboratory abnormalities) suffered from lactose intolerance.\textsuperscript{553} A recent study revealed that lactose free-diet markedly attenuated itch severity in up to 64% of these patients.\textsuperscript{554} Thus, in patients with chronic pruritus of unknown origin a H\textsubscript{2}-exhalation test should be included in the diagnostic work-up.

\textit{Celiac disease}

Celiac disease is caused by an autoimmune reaction of the body against the gliadin glycoprotein of the gluten protein which is present in wheat and other cereals. The disorder is accompanied by gastrointestinal changes leading to malabsorption-related changes such as iron deficiency anaemia, vitamin deficiency and secondary hyperparathyroidism due to reduce calcium and vitamin D absorption. Furthermore, celiac disease is associated with other disorders such as dermatitis herpetiformis and primary biliary cirrhosis although the underlying mechanism is unclear.\textsuperscript{555} Generalized pruritus may be seen in celiac patients which could be ascribed to various factors including iron deficiency, secondary hyperparathyroidism or associated diseases such as dermatitis herpetiformis and primary biliary cirrhosis.\textsuperscript{556} Interestingly, in patients with celiac-disease associated cholangitis pruritus resolved and liver serum tests improved after the initiation of a gluten-free
diet.\textsuperscript{555,557} Thus, pruritogenic cytokines released from immune cells may cause itch sensations in these patients.

**SOLID TUMOURS**

Patients with solid tumours rarely report of generalized pruritus and the incidence seems to be less than 1\%.\textsuperscript{558} Generalized pruritus has been reported in case reports, case series and larger studies in patients with thyroid carcinoma,\textsuperscript{559} laryngeal cancer,\textsuperscript{560,561} metastasized squamous cell carcinoma of the tongue,\textsuperscript{562} lung cancer,\textsuperscript{321,559,563-565} (metastasized) breast cancer,\textsuperscript{564,565} stomach cancer,\textsuperscript{561,565} pancreas cancer,\textsuperscript{566} metastatic colon cancer,\textsuperscript{564} sarcoma,\textsuperscript{567} uterine cancer,\textsuperscript{564} prostate,\textsuperscript{568} and renal cancer.\textsuperscript{565} Pruritus may precede the diagnosis of malignancy up to several years.\textsuperscript{561,565} One study investigating 125 patients with generalized pruritus were followed over a time period of six years. Malignancy was diagnosed in eight patients, among most were lymphoma.\textsuperscript{565} Also in several other studies lymphoma appeared to be higher represented as a cause for pruritus compared to solid tumours.\textsuperscript{321,559,566,568}

Besides generalized pruritus may also be localized and is then typically experienced on the extensor surfaces of the upper extremities, shoulders, upper thorax, inner areas of the thighs and pretibial.\textsuperscript{313} Certain malignancy cause specific localized itching as observed in prostate cancer as scrotal itch, in anal, rectal, and sigmoidal cancer as peri-anal pruritus, in vulva and cervical cancer as vulval itch, and in advanced brain tumours as itching restricted to the nostrils.\textsuperscript{313,569-571} In general, pruritus rapidly resolves if the tumour is removed after surgery or successfully treated by chemo- and/or radiotherapy.\textsuperscript{313,563,572} If pruritus reappears this may be a sign of tumour recurrence and formation of metastasis.\textsuperscript{563,572} The pathogenesis of pruritus in solid tumours is unknown but may be due to (i) localized invasion of the malignancy associated with compression of sensory nerve fibers, (ii) substances or metabolites released from necrotic tumour cells, (iii) immune reaction against cutaneous micrometastasis, (iv) obstruction of the bile duct system causing cholestasis, (v) xerosis cutis, and (vi) neuropathic itch as an adverse effect of anti-tumour therapy.\textsuperscript{313,316,571}
INFECTIOUS DISEASES

**Human immunodeficiency virus**

The majority of patients infected with the human immunodeficiency virus (HIV) will suffer from pruritus during their course of disease. Pruritus may be caused by typical HIV-associated skin disorders such as, papulosquamous disorders, skin infections including scabies, photodermatitis, xerosis, drug reactions, and lymphoproliferative disorders. However, itching is also seen in the absence of any HIV-associated skin disorder and may be the presenting symptom of this infection. Furthermore, itch severity might intensify as the disease progresses. Notably, with advance of the disease a switch from TH1- to TH2-cytokines with increased levels of IL-4, IL-5 and IL-10 was observed. Interestingly, HIV-patients with pruritus had higher serum levels cytokines such as TNF-α and IL-10.

The underlying mechanisms of generalized pruritus in patients with HIV-infections remain unknown, but may be related to (i) increased serum levels of pruritogenic cytokines, (ii) cytokine-induced synthesis of PGE₂, (iii) hypereosinophilia and hyperimmunogloulinaemia E observed in patients with intractable pruritus, and (iv) neuropathy due to disease mechanism and the neurotoxic anti-retroviral therapy.

**Varicella zoster virus**

 Reactivation of the varicella zoster virus (shingles) is known as herpes zoster. The best known complication of herpes zoster is postherpetic neuralgia in the affected dermatoma, however, many patients also suffer from localized postherpetic pruritus. Postherpetic pruritus is presumably caused by unprovoked firing of the peripheral and/or central neurons that mediate itch sensation. It has been reported that postherpetic itch occurring in neurons innervating skin left severely deafferented from shingles, so-called numbs, patients can give themselves painless injuries from chronic scratching.
Other infections

Various other, mainly viral infections have been linked to onset of pruritus among which were hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus, and dengue fever virus. Except for HBV and HCV infections, pruritus is typically observed in those patients presenting with cutaneous manifestations of these viral infections.581

Pruritus in the elderly

Itching is a frequent complaint of the elderly population defined as individuals aged above 65 years and might be caused by various cutaneous or systemic diseases.582 Aged skin is characterized by atrophy, reduced skin barrier function and most importantly dryness due to lower water content.582,583 Xerosis cutis induces the expression of pruritogenic cytokines in the skin and may thereby elicit pruritus.540 For further facts about senile itching the reader is referred to the recent review of Reich et al.582

Drug-induced pruritus

Acute and chronic pruritus is a common adverse effect of various drugs.3 Thus, beside physical examination and laboratory analysis, a proper medication analysis should be performed in every patient with pruritus of unknown origin. The molecular mechanisms of the anti-malaria drug chloroquine have been resolved in part as discussed above. For most other drugs – in particular those causing chronic pruritus – the involved pathophysiological mechanisms remain unclear. For detailed information about drug-induced pruritus the reader is referred to the review of Reich and colleagues.3
CONCLUSION

Pruritus research has largely emerged over the past few years and important advances in the understanding of the peripheral receptors, intracellular signalling pathways, cells, circuitries and central transmitters have been made. Many receptors on sensory neurons are capable of mediating exogenous and endogenous chemical itch signals among which are MrgX, MrgD and likely other Mrg subtypes, H1, H4, 5-HT2, PAR4, ET-A and B1/B2. These GPCRs appear to relay to downstream effector channels such as TRPV1 and TRPA1 which are required for translation into action potentials in these neurons. On spinal cord level transmitters of the itch pathways include glutamate, Nppb, GRP and neuromedin B. The discovery of these novel therapeutic targets has opened new avenues for development of causative treatment strategies for this agonizing symptom. The relevance of these receptors and signalling molecules in chronic pruritus of systemic diseases, however, still remains to be elucidated.
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