Advances in TRP channel drug discovery: from target validation to clinical studies

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Abstract | Transient receptor potential (TRP) channels are multifunctional signalling molecules with many roles in sensory perception and cellular physiology. Therefore, it is not surprising that TRP channels have been implicated in numerous diseases, including hereditary disorders caused by defects in genes encoding TRP channels (TRP channelopathies). Most TRP channels are located at the cell surface, which makes them generally accessible drug targets. Early drug discovery efforts to target TRP channels focused on pain, but as our knowledge of TRP channels and their role in health and disease has grown, these efforts have expanded into new clinical indications, ranging from respiratory disorders through neurological and psychiatric diseases to diabetes and cancer. In this Review, we discuss recent findings in TRP channel structural biology that can affect both drug development and clinical indications. We also discuss the clinical promise of novel TRP channel modulators, aimed at both established and emerging targets. Last, we address the challenges that these compounds may face in clinical practice, including the need for carefully targeted approaches to minimize potential side-effects due to the multifunctional roles of TRP channels.

In 1969, a *Drosophila* mutant with defective light sensing was identified, which showed only a transient receptor potential (TRP) when exposed to continuous light instead of the expected sustained response[1]. This was later found to be caused by the lack of a functional copy of the gene coding for an ion channel, which was named *trp*[2]. However, the name 'TRP' channel is really a misnomer as the wild-type channel in fact causes a persistent (and not transient) current. This behaviour of TRPs is in contrast to many ion channels, which fully adapt when exposed to constant stimulation.

TRPs are multifunctional signalling molecules, expressed in many tissues and cell types[3,4]. Most TRPs are polymodal channels, so-called coincidence detectors that are activated by both physical (temperature, voltage, pressure and tension) and chemical stimuli[4]. Beyond that, few generalizations can be made about TRP channels. Some TRPs function as non-selective cation channels in the plasma membrane; others regulate Ca^{2+} release in intracellular organelles.

The mammalian TRP channel superfamily has 28 members (27 in humans)[3]. On the basis of sequence homology, the superfamily is divided into six subfamilies: canonical (also known as short TRPs, TRPC1–7), vanilloid (also known as TRP channel subfamily V, TRPV1–6), melastatin (also known as TRP channel subfamily M, TRPM1–8), ankyrin (also known as TRP channel subfamily A, TRPA1), mucolipins (TRPML1–3), and polycystins (also known as polycystic kidney disease 2-like 1 protein (PKD2L1, also termed TRPP3) and polycystin-2 (TRPP2))[3,4]. Because these subfamilies were created based on sequence homology and not function, members often have little in common. For example, TRPM2 is a redox sensor in macrophages[5]; TRPM7 provides a major Mg^{2+} uptake pathway in intestinal epithelial cells[6]; and TRPM8 detects cold and menthol in sensory neurons[7,8], but regulates epithelial growth in response to androgens in the prostate[9]. Despite the structural similarities shared by these proteins (FIG. 1; BOX 1), there are enough differences to develop subtype-selective compounds.

Most TRPs have restricted expression patterns, but their varied tissue distribution means that the superfamily affects most cells, tissues and organs of the human body. Overall, the diverse physiological functions and regulatory mechanisms of TRPs affect how they are implicated in disease. These include both genetic and acquired channelopathies, as well as many disorders in which targeting one or more TRP channel could alleviate symptoms or provide therapeutic effects[4].

Most TRPs are subjects of intensive drug discovery and development efforts. In this Review, we summarize the crucial advances of the past decade in our

Coincidence detector
A process by which a neuron can detect and converge separate signals into one input (such as an action potential).

Gustatory sweating
Perspiration in the head-and-neck area after eating hot spicy food.

understanding of the complex roles that TRPs have in the development and progression of human disease. Whereas our improved understanding of the structures of TRP channels will undoubtedly aid drug discovery (BOX 1), the increasingly diverse physiological roles of TRPs pose a serious challenge to drug development. Indeed, it has proved difficult to obtain sufficient specificity for clinically useful intervention without unacceptable side effects. Although developing clinically useful TRP modulator drugs is challenging, the potential rewards are enormous given the pathogenic role of TRPs in chronic pain, neurology, oncology, dermatology, pulmonology, cardiology, urology and rare diseases.

TRP channel biology and pathology

Although all TRP channels are evolutionarily highly conserved, their sensitivity to external stimuli show striking species-related differences. For example, TRPV1 is activated by capsaicin in mammals (it was originally cloned as the 'capsaicin receptor')[10], but not in birds[11]. However, changing position 578 in the S4/S5 helix of *cTrpv1* from alanine to glutamine renders the chicken receptor capsaicin-sensitive[12]. TRPV1 also shows distinct, species-dependent, heat-activation thresholds, and so TRPV1 is a noxious heat sensor in some mammals (including humans)[13] but not in others (for example, camels that have evolutionarily adapted to desert heat)[14]. Similarly, the sensitivity of TRPA1 to cold differs between rodents and primates[15]. These species-dependent differences in channel sensitivity and function should be considered when selecting experimental animal models and interpreting the results.

TRPV1 is a prime example of diversity in TRP channel expression and function. TRPV1 is highly expressed on primary sensory neurons as a major integrator of painful stimuli (afferent function) and a key initiator of neurogenic inflammation (efferent function)[16] (FIG. 2). Moreover, TRPV1-expressing sensory neurons have been implicated in warmth sensing[17,18] and itching[19]. In the viscera, neuronal TRPV1 triggers reflex pathways like cough, emesis, heart rate, micturition and intestinal peristalsis[16]. Albeit at much lower levels, TRPV1 is also expressed in various brain nuclei[20] and in non-neuronal cells[21].

TRPV1 is unique among drug targets in that its initial excitation by agonists is followed by a lasting refractory state (traditionally referred to as desensitization) in which TRPV1-expressing neurons are not responsive to both a repeated capsaicin challenge and to various unrelated stimuli[16] (FIG. 2).

The role of TRPV1 in thermoregulation is well established[22,23]. In rodents, activation of TRPV1 with capsaicin initiates heat-loss behaviour at warm ambient temperatures (30–32.5 °C) and mice exhibit 'red ear' caused by vasodilation and seek the cool surface of the cage[16]. By contrast, TRPV1-deficient ($\mathit{Trpv1}^{-/-}$ or capsaicin-desensitized) mice display deficiencies in heat-loss mechanisms (such as body licking) and develop hyperthermia when exposed to 35 °C (REF.[24]). In humans, capsaicin may cause gustatory sweating as a mechanism of heat loss[16], whereas TRPV1 antagonists can increase or decrease body temperature, or even leave it unchanged ('thermoneutral antagonists')[25–27]. These thermoregulatory side effects can be exploited for pharmacotherapy: TRPV1 antagonists that cause hyperthermia as a dose-limiting, on-target adverse effect may also help to restore normal body temperature after medical cooling[28,29]. In turn, TRPV1 agonists

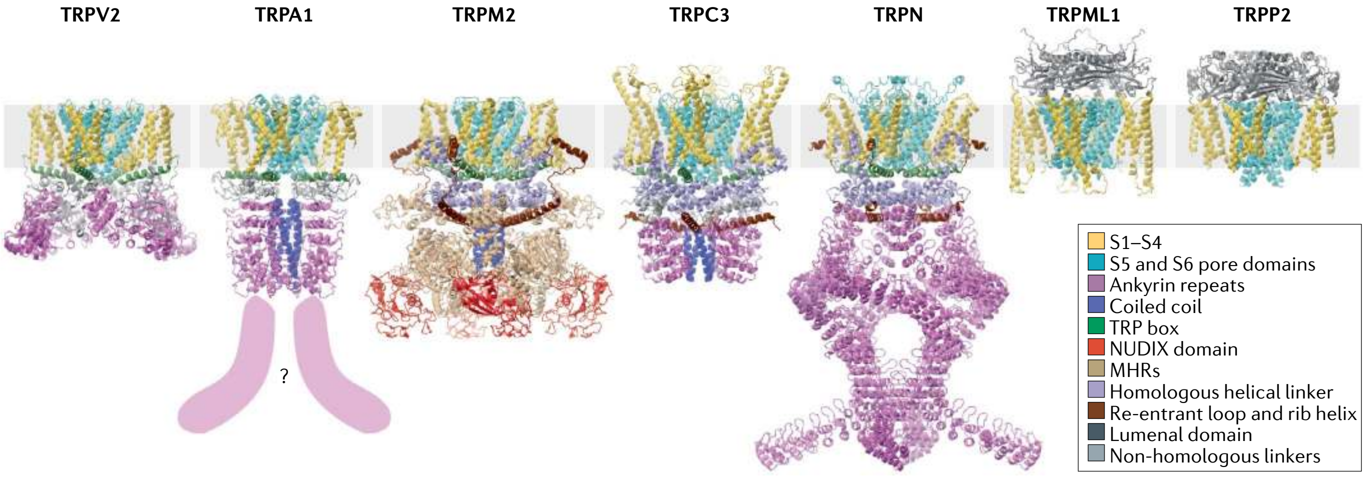


Fig. 1 | **Similarities and differences between the structures of TRP channels.** Representative structures for each TRP channel subfamily, coloured to highlight common structural features. The S1–S4 (gold) and the S5 and S6 pore domains (cyan) are the only domains common to all subfamilies. The TRPA, TRPV, TRPM, TRPC and TRPN channels have TRP box helices (dark green). The TRPA, TRPC, TRPN and TRPV channels have amino (N)-terminal cytoplasmic ankyrin repeats (violet; the TRPA1 structure is missing about 11 repeats, as indicated by the question mark and violet shapes). The TRPC, TRPM and TRPN channels have a homologous pre-S1 helical linker (lilac) and C-terminal re-entrant loop and rib helix (brown), which is followed by a coiled coil in the TRPC and TRPM channels (blue; TRPA channels also have a carboxy (C)-terminal coiled coil). The melastatin homology regions (MHRs) of TRPM channels are shown in tan, and the NUDIX domain unique to TRPM2 is shown in red. The TRPML and TRPP channels have a homologous lumenal domain (dark grey). The pre-S1 linkers of the TRPV and TRPA channels and the C-terminal region of the TRPV channels (light grey) share no clear homology outside their respective subfamilies. The structures depicted are human TRPA1 (PDB ID 3J9P), human TRPC3 (PDB ID 6CUD), human TRPM2 (PDB ID 6MIX), *Drosophila* TRPN (NompC; PDB ID 5VKQ), human TRPP2 (PDB ID 5T4D), human TRPML1 (PDB ID 5JW5) and rabbit TRPV2 (PDB ID 6OO3).

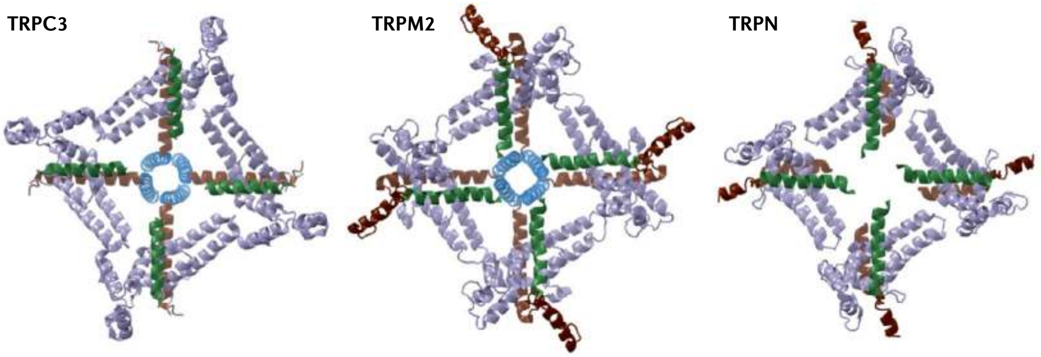
Box 1 | **Emerging features in TRP channel structures**

Effective drug discovery targeting individual TRP channels will need in-depth knowledge of the druggable structural sites and the activation and inactivation mechanisms. This, in turn, requires high-resolution structures in multiple states combined with functional studies. However, drug discovery strategies can also be conceptually enhanced by leveraging the rapidly multiplying structural information to take a bird's-eye view of the whole family.

With at least one structure available for each subfamily, we can now identify several recurring structural features. As predicted from their sequence homology to voltage-gated channels, all TRP channels have the S1–S4 transmembrane segments that form peripheral sensing domains (yellow in FIG. 1), whereas S5 and S6 tetramerize to create a central pore (cyan in FIG. 1). Other recurring structural features became apparent from sequence analyses, such as the ankyrin repeats in the N-terminal cytosolic domains of the TRPA, TRPC, TRPV and nonmammalian TRPN channels[293] (pink in FIG. 1). Moreover, the TRP box, a sequence motif first detected in the TRPC, TRPM and TRPV channels, forms a helix parallel to the membrane (green in FIG. 1). This helix, which is a link between the cytosolic and transmembrane domains, is actually found in all TRP subfamilies except TRPML and TRPP.

Additional unanticipated homologous structural features are shared between the TRPC, TRPM and nonmammalian TRPN channels. In the BOX 1 figure below, these shared features are viewed from above the membrane to better visualize their common structural fold, with the green TRP box helix included for reference. First, the approximately 150 N-terminal residues preceding the transmembrane domain (lilac) form a cytosolic helical platform and a re-entrant loop that penetrates the inner leaflet of the membrane near the S1–S4 domain. Second, the approximately 80–100 C-terminal residues following the TRP box helix (green) also form a re-entrant loop followed by a long helix parallel to the membrane plane (brown). This long helix, named the 'rib helix' in TRPM channels[294], 'connecting helix' in TRPC channels[295] and 'CH2' in TRPN channels[296], is then followed by a coiled coil in TRPC and TRPM channels (blue).

Shared structural features between TRP channel subfamilies like the ones described here suggest shared regulatory mechanisms. Thus, information gained in individual subfamilies can and should be mined to advance our understanding of channels from other subfamilies with similar structural features.



that cause hypothermia may protect the brain after stroke[30]. As reviewed elsewhere[16,22,23], there is no consensus in the literature as to the exact anatomic site of the TRPV1-expressing thermoregulatory centre, or the mechanism by which it regulates body temperature.

Cryo-electron microscopy and X-ray crystallography have provided novel insights into TRP channel structure and function and highlighted several druggable sites (FIG. 3). TRPV1 has fourfold symmetry with different pore profiles for ligand-bound structures[31], and a vanilloid-binding pocket deep within the membrane bilayer[32] (FIG. 3b,f). TRPA1 is a sentinel for electrophilic irritants and has a distinct allosteric nexus where a covalent modification of cysteine residues regulates channel activity[33] (FIG. 3c). Indeed, all known synthetic TRPA1 antagonists, such as A-967079, act as negative allosteric modulators (FIG. 3b). This is important because they can block TRPA1 (over)activation, and yet leave some level of physiological activity intact, in contrast to traditional orthosteric antagonists.

Epigenetic regulation of TRPs is an emerging area of research. In rats, histone H3 acetylation at the *Trpv1* promoter region leads to increased TRPV1 protein expression in sensory neurons with concomitant visceral hyperalgesia[34]. In mice, SUMOylation protects TRPV1 from metabolic damage during experimental diabetes and thus delays the development of neuropathic pain[35]. TRPV1 from human donor tissue is also SUMOylated[35]. Likewise, methylation of the human *TRPA1* promoter region can result in increased TRPA1 levels and altered pain perception[36]. Indeed, *TRPA1* gene methylation is dysregulated in patients with Crohn disease, contributing to visceral pain[37].

Genetic defects in TRPs — or TRP channelopathies — are increasingly recognized causes of hereditary human disease[4]. For example, TRPV4 channelopathy[38] is linked to at least nine different diseases, ranging from autosomal dominant brachyolmia type 3 to Charcot–Marie–Tooth neuropathy type 2. Furthermore, polymorphisms in TRP genes may regulate disease risk, as exemplified by the reduced migraine incidence in carriers of rs10166942, which correlates to reduced *TRPM8* gene expression[39].

Pain: TRP channels as analgesic targets

Medical control of chronic pain is frequently unsatisfactory, and the current therapeutic pain market remains dominated by agents that have been around for decades. Narcotics (opioids) are effective painkillers, but, acting in the brain, they are also addictive. A logical strategy to

Allosteric nexus
A substructure in a protein that serves as a shared regulatory site, binding to chemical ligands or responding to physiological stimuli, and resulting in changes in the shape and activity of the protein.

SUMOylation
Covalent modification by the small ubiquitin-related modifier peptide.

Brachyolmia type 3
A form of severe skeletal dysplasia characterized by an abnormal curve of the spine (kyphoscoliosis) and flattened cervical vertebrae.

Charcot–Marie–Tooth neuropathy type 2
A genetic defect that causes decreased heat, cold and touch sensations mostly in the hands and feet owing to axon damage.

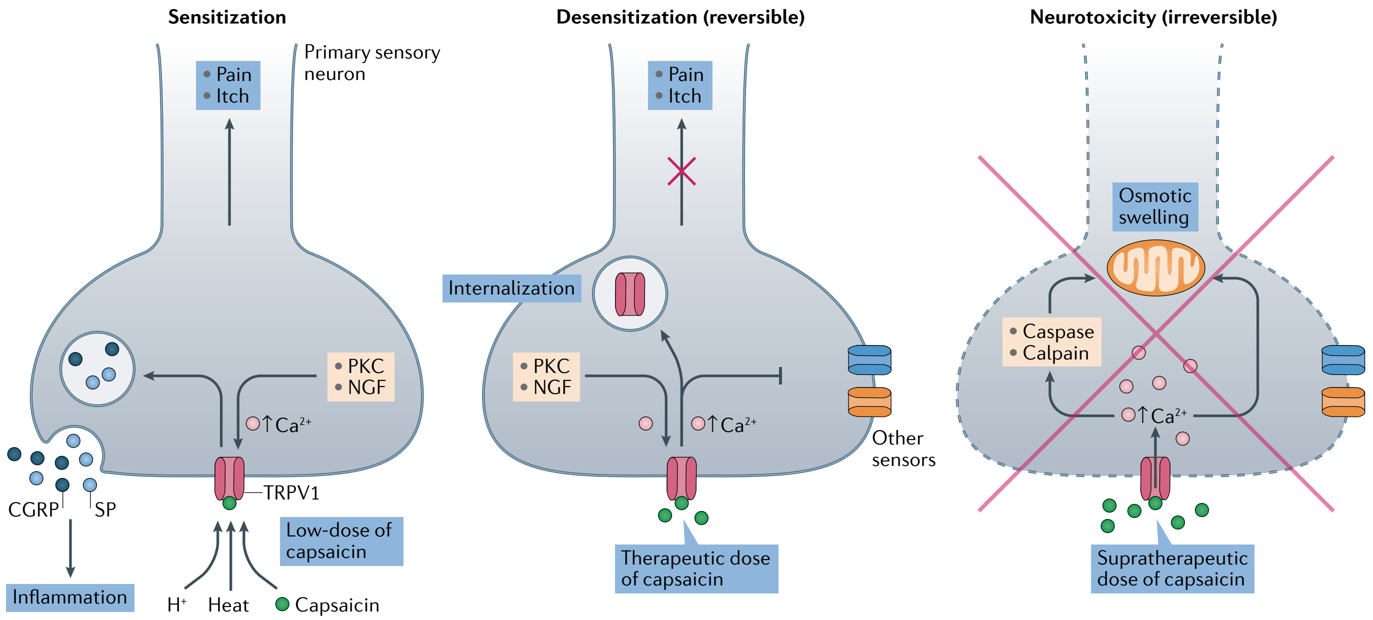


Fig. 2 | **TRPV1 in the pain pathway: similarities and differences between desensitization by agonists and blockade by antagonists.** TRPV1 is expressed on primary sensory neurons of the unmyelinated (C fibre) type. Activation of TRPV1 by agonists like capsaicin, heat and protons (and putative 'endovanilloids') triggers the release of pro-inflammatory neuropeptides like substance P (SP) and calcitonin gene-related peptide (CGRP), thereby initiating the biochemical cascade known as neurogenic inflammation. At the same time, an impulse is generated that is perceived in the brain as pain or itching. Protein kinase C (PKC) and nerve growth factor (NGF) lower the activation threshold of TRPV1 (sensitization). Stimulation of TRPV1 with a therapeutic dose of capsaicin results in a lasting (up to months) but fully reversible state in which the previously excited neurons remain unresponsive ('silent') to further challenge; traditionally, this is called desensitization. High-dose capsaicin patches and site-specific injections relieve pain by this mechanism. There is an ill-defined line between reversible desensitization and irreversible neurotoxicity achieved by therapeutic and supratherapeutic doses of capsaicin, respectively. Although both desensitization and neurotoxicity depend on capsaicin-induced Ca^{2+} influx through the TRPV1 channel, the downstream molecular mechanisms are still poorly understood. Mitochondrium swelling is an early ultrastructural sign of capsaicin neurotoxicity. Ca^{2+} is thought to sequester in mitochondria, where it triggers apoptosis using a molecular pathway that includes caspase activation. Intrathecal resiniferatoxin is used as a 'molecular scalpel' to achieve permanent analgesia in cancer patients with chronic, intractable pain by ablating sensory neurons. In principle, a similar strategy can be used to kill cancer cells that overexpress TRPV1 compared with normal counterparts. We note that small molecule TRPV1 antagonists prevent only TRPV1 activation by agonists, leaving other pain sensors intact.

avoid opioid side effects is to target the beginning of the pain pathway: the nociceptor where pain is generated[25,40]. Hence there is tremendous interest expressed by pharmaceutical companies in TRP channels that detect noxious stimuli in the periphery (FIG. 4).

The 'capsaicin receptor', TRPV1. Desensitization to capsaicin has a clear therapeutic potential (FIG. 2). Indeed, high-dose capsaicin patches[41,42] and site-specific injections[43] are clinically proven to provide meaningful pain relief in patients with osteoarthritis, post-herpetic neuralgia and diabetic polyneuropathy. Peripheral expression of TRPV1 on nociceptive fibres is predictive of the patient's response to capsaicin therapy, which may explain the discrepant outcome in clinical trials with capsaicin in patients with diabetic polyneuropathy in whom nociceptive fibres often degenerate during advanced disease[44].

Capsaicin evokes an intense initial pain reaction that limits the dose that patients can tolerate[16]. To reduce this adverse effect, 'non-pungent' TRPV1 agonists like olvanil (NE19550)[45] and MRD-652 (REF.[46]) have been developed that differ from capsaicin in the activation kinetics of the receptor. These compounds showed promise in animal models of pain[45,46], but their clinical value remains to be demonstrated.

The ultrapotent capsaicin analogue, resiniferatoxin, is undergoing clinical trials as a 'molecular scalpel' with which to achieve permanent analgesia in severe osteoarthritic pain[47,48], and in cancer patients with chronic intractable pain[49]. This approach has already succeeded in the veterinary clinic, in which intrathecal resiniferatoxin provided lasting pain relief and restored ambulation in dogs with osteosarcoma[50]. Resiniferatoxin has been tested in a small number of female patients with cervical cancer metastatic to pelvic bone, and the results are promising so far[51].

Because TRPV1 agonists that cause desensitization can be perceived as functional antagonists of the receptor (FIG. 2), it has been postulated that small molecule TRPV1 antagonists can be also pursued as therapeutic agents[25,52,53]. Indeed, TRPV1 is a highly druggable target[52]. Various antagonist chemotypes have been discovered, and many have matured into clinical lead molecules[25,52,53]. The efficacy of TRPV1 antagonists in preclinical pain models varied; some showed efficacy in both inflammatory (for example, complete Freund adjuvant-induced arthritic pain) and neuropathic pain models (such as the Chung model), whereas others were active only in the inflammation models[25,53]. We note that both the magnitude of the analgesic effect and the dose needed to demonstrate efficacy varied.

Freund adjuvant-induced arthritic pain
A frequently used rodent model for screening analgesics against inflammatory pain induced by local injection of dead mycobacteria.

Chung model
A frequently used model for analgesic screening against neuropathic pain induced by unilateral spinal nerve ligation.

When using hypothermia by capsaicin as an on-target engagement model, many TRPV1 antagonists actually caused the opposite effect, hyperthermia[25]. However, this febrile reaction could be managed with simple antipyretic agents like acetaminophen and disappeared upon repeated dosing, paving the way to studies in human volunteers[54]. Since *Trpv1*$^{-/-}$ mice are less responsive to noxious heat[55,56], and rodents desensitized to capsaicin show dramatically increased noxious heat threshold in the hot plate test[16], it was unsurprising that TRPV1 antagonists impaired the cutaneous noxious heat sensation in humans, leading to burn injuries as a common adverse effect[53].

Owing to these unacceptable on-target adverse effects, many first-generation TRPV1 antagonists were withdrawn from clinical trials: some, like AMG517, because of febrile reactions[57] and others, like MK2295, because of the burn injuries[58]. Some antagonists that progressed into phase II efficacy trials failed to demonstrate analgesic activity, such as a terminated trial of AZD1386 for osteoarthritic pain[59]. Other clinical trials were terminated without explanation, such as those sponsored by Sanofi/Glenmark (GRC-6211) and PharmEste (PHE575). Since functional TRPV1 expression on nociceptors predicts the patient's response to agonist (capsaicin) treatment[42], one may argue that TRPV1 antagonist trial participants should also be selected on the basis of their capsaicin sensitivity. It is also worth mentioning that vitamin D was recently shown to act as a partial agonist of TRPV1 at physiologically relevant free plasma concentrations[60].

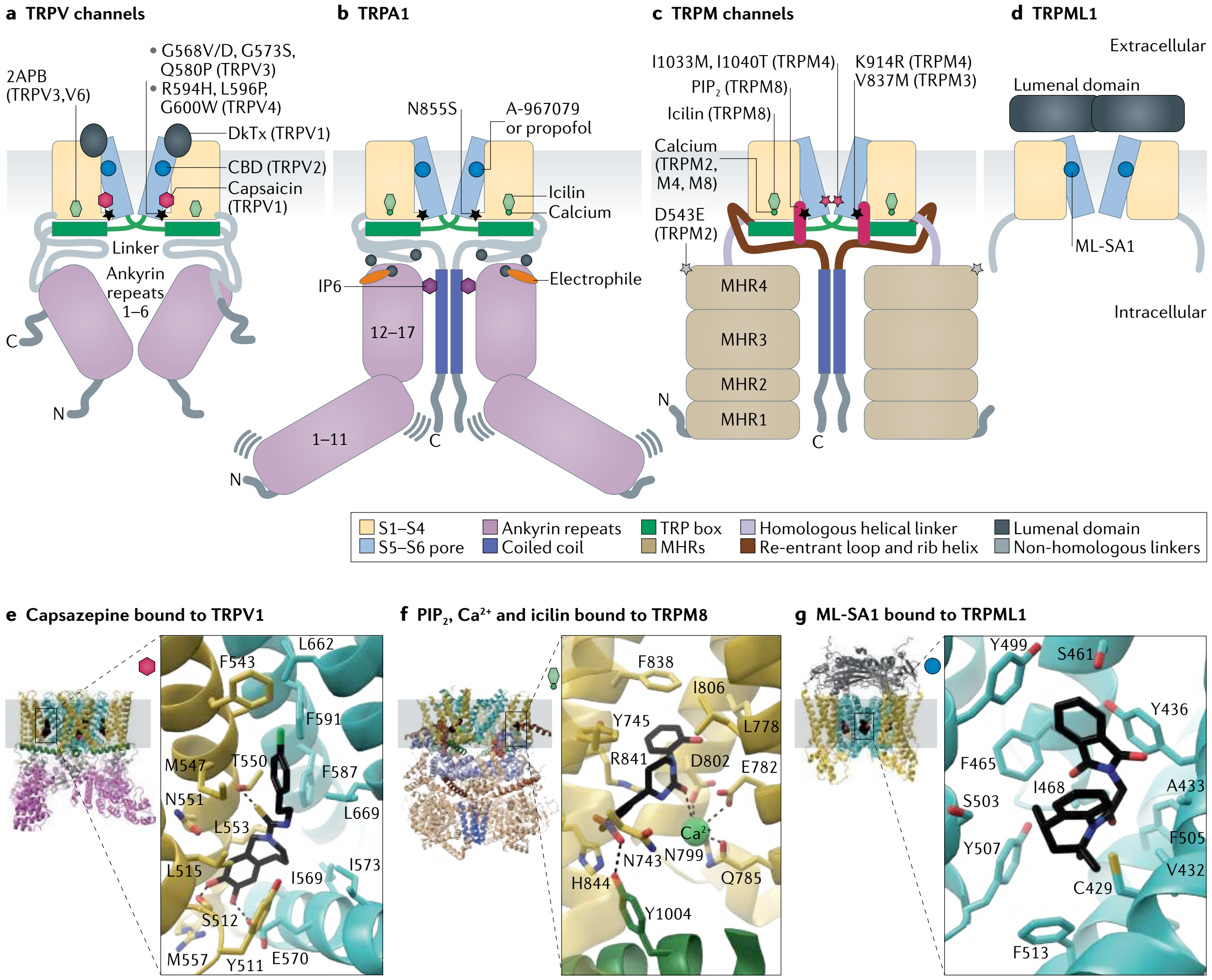


Fig. 3 | **Structural information on ligand and drug binding by TRP channels, and gain-of-function mutations.** Protein colouring is the same as in FIG. 1. **a**–**d** | Schematic descriptions of TRPV (**a**), TRPA1 (**b**), TRPM (**c**) and TRPM1 (**d**) illustrate the general binding site locations identified in structural findings of TRP channels. Similar ligand shapes indicate similar ligand-binding site locations in the respective proteins, and example ligands are labelled. Black stars indicate mutations associated with human diseases that are located at the elbow connection between the S4–S5 linker and the S5 helix, and magenta stars in the S6 helix pore gate indicate mutations that lead to hypersensitive or constitutively active channels and gain-of-function phenotypes. Grey stars mark the TRPM2 D543E hypoactive mutation. **e** | Sample ligand-binding sites from the antagonist capsazepine bound to rat TRPV1 (PDB ID 5IS0). **f** | Co-agonists PIP2, Ca^{2+} and icilin bound to *Ficedula albicollis* TRPM8 (PDB ID 6NR3). **g** | Agonist ML-SA1 bound to human TRPML1 (PDB ID 6E7Z).

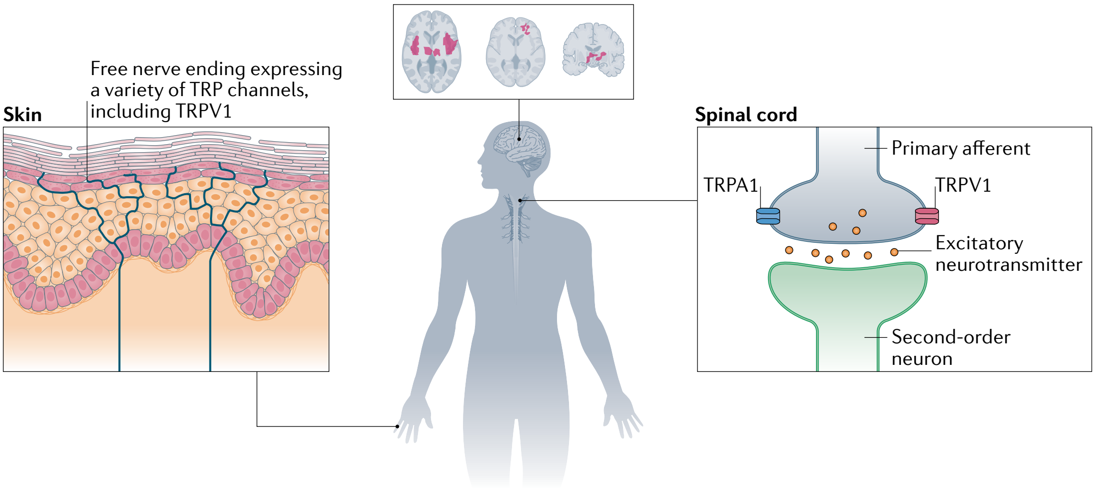


Fig. 4 | **The pain pathway: wide expression of TRP channels in sensory neurons, skin and brain.** Several TRP channels in free nerve endings or sensory corpuscles of sensory neurons transduce painful thermal (TRPV1, TRPA1, TRPM8 and TRPM3), chemical (TRPA1) and mechanical (TRPC1, TRPC3, TRPC6, TRPA1, TRPV2 and TRPV4) stimuli into electrophysiological excitation. TRP channels that do not participate in physiological sensation, but are recruited under pathophysiological conditions (for example, TRPC5 in mechanosensation) are of particular interest. TRP channels in keratinocytes, satellite glia and Schwann cells (TRPV1, TRPA1) also participate in transduction of painful stimuli. TRP channels in the central terminals of sensory neurons amplify and modulate neurotransmitter release in the spinal cord. TRP channels in the brain play critical parts in several physiological processes. It is likely that different TRP channels in the brain have different roles in acute nociceptive versus maladaptive chronic pain. Pain representation in the brain shifts from from nociceptive circuits in acute pain (left brain scan) to emotional circuits during chronic pain in functional magnetic resonance imaging (middle and right brain scans). Therefore, an assessment of central action or peripheral restriction of a TRP channel modulator needs to be carefully performed to maximize efficacy and therapeutic window.

A partial agonist can either act as an agonist or antagonist, depending on the presence of other ligands. Such effects may contribute to the variability of TRPV1 antagonists in clinical trials.

Although these problems tempered expectations, and many companies abandoned TRPV1 as an analgesic target, there have been a few promising developments. For example, the second-generation molecule, JNJ-39439335 (mavatrep), showed significant improvement versus placebo in stair climbing-induced clinical pain in participants with knee osteoarthritis[61]. Another compound, NEO6860, also showed an analgesic trend, although it did not statistically outperform placebo, without affecting body temperature or heat pain perception[62]. It remains to be seen whether these molecules — or other 'thermoneutral' antagonists, like GRTE16523 (REF.[63]) — can demonstrate meaningful analgesic activity in the clinic. Ultimately, it will probably be easier to dissociate beneficial heat sensing from therapeutic inhibition of TRPV1 activity by targeted antagonist delivery because most, if not all, inhibitors of TRPV1 activation will inhibit heat sensing.

The role of TRPV1 phosphorylation by protein kinase-C (PKC) in the development of inflammatory hyperalgesia is well established[64]. Eliminating the PKC phosphorylation site S801 by CRISPR/Cas9 editing in TRPV1 reduced the ongoing pain caused by masseter muscle inflammation without blocking physiological TRPV1 functions[65]. This observation raises the possibility of engineering novel TRPV1 antagonists that selectively interact at the phosphorylated TRPV1.

As to future clinical testing, a promising indication for TRPV1 analgesia is gout. In experimental animals, urate crystals activate TRPV1, and the resultant pain is blocked by both genetic inactivation (*Trpv1*$^{-/-}$ mice) and pharmacological blockade (SB-366791)[66]. In a mouse model of gout arthritis, eucalyptol reduced both inflammation and pain by preventing the urate-induced up-regulation of TRPV1 expression in ankle tissues[67]. Furthermore, SB-366791 attenuates dental pain in rats[68], and, as an added benefit, prevents alveolar bone loss in a rat model of periodontal disease[69]. Other potential indications with increased *TRPV1* mRNA levels include endometriosis[70] and chronic lower back pain[71]. However, because much of the earlier preclinical efficacy data that predicted clinical value in inflammatory pain (such as osteoarthritic pain) did not translate in patients, caution is advised for these new potential indications.

The chemical nocisensor, TRPA1. *TRPA1* gene variants have been linked to paradoxical heat (painful cold) sensation[72], sickle cell pain crisis[73], carbamazepine-responsive cramp-fasciculation syndrome[74], and familial episodic pain syndrome (FEPS)[75]. FEPS is a gain-of-function TRPA1 channelopathy that causes debilitating upper body pain due to increased inward currents in response to TRPA1 channel activation at normal resting potentials. From a clinical perspective, this observation is not easy to interpret as patients carry both mutant (human *TRPA1* N855S) and wild-type alleles. TRPA1 is a tetramer and it is reasonable to assume that

CRISPR/Cas9 editing
Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) is a technology that allows genetic material to be added, removed or altered by creating a 'guide' RNA to target specific DNA sequences.

Carbamazepine-responsive cramp-fasciculation syndrome
Peripheral nerve hyperexcitability syndrome that presents with stiffness, muscle pain, cramps and exercise intolerance.

Familial episodic pain syndrome
(FEPS). Rare genetic peripheral neuropathy disorder characterized by recurrent, intense upper body or lower limb pain in response to fatigue, fasting, physical stress or cold exposure.

patients express various mixtures of normal and mutant channels[76]. Although one cannot predict the properties of these heteromultimers, the N855S mutation, like the analogous gain-of-function mutations in *TRPV4* and *TRPM4*, is in the linker that moves during channel gating (FIG. 3b–d), explaining how it causes channels to open more readily. An antagonist that selectively blocks the mutant FEPS protein would be optimal, but companies are unlikely to invest resources into such a small market.

Genetic deletion or pharmacological blockade of TRPA1 vastly attenuate responses to many harmful chemical stimuli, ranging from formaldehyde, through acrolein (present in cigarette smoke) and diesel exhaust, to tear gases[77,78]. Moreover, endogenous compounds like methylglyoxal[79,80] (a product of aberrant glucose metabolism) and reactive oxygen species (ROS) and nitrogen species all converge on TRPA1 (REF.[81]): these electrophilic compounds can activate the channel after covalently modifying a hypersensitive cysteine in the cytoplasmic domain of TRPA1 by electrophilic attack[82] (FIG. 3c).

The temperature (cold) sensitivity of TRPA1 is subject to much debate and will not be discussed here, except to mention that *Trpa1*$^{-/-}$ mice show attenuated cold allodynia evoked by anticancer drugs like oxaliplatin[83], and by ischaemia and reperfusion injury of the rodent hindlimb, a murine model of complex regional pain syndrome type I (CRPS-I)[84]. Importantly, TRPA1 antagonists recapitulated the effects of genetic *Trpa1* inactivation[84], indicating a novel therapeutic strategy in CRPS-I patients. *Trpa1*$^{-/-}$ mice also displayed reduced visceromotor responses to colorectal distension[85], indicating a role for TRPA1 in mechanical pain; and *Trpa1*$^{-/-}$ rats showed decreased pain behaviours in response to chemical agonists of TRPA1, but normal responses to other pain stimuli, including cold, and itching[86]. Animal studies with TRPA1 antagonists (such as A-967079, HC-030031 and AMG0902) suggested therapeutic potential in patients with neuropathic pain[87].

We note that CHEM-5861528, when given with streptozotocin to rats, ameliorated the pain and reduced the loss of intraepidermal nerve fibres[88]. If these results were to be confirmed in diabetic patients, this observation suggests that TRPA1 antagonists can be disease-modifying by preventing (or at least delaying) the development of diabetic polyneuropathy.

As of today, only one TRPA1 antagonist has completed phase II clinical trials, Glenmark's GRC17536[89]. Although GRC17536 significantly reduced pain scores in the non-denervation group of patients with painful diabetic polyneuropathy without worrisome side effects, the compound had problems with bioavailability/pharmacokinetics and did not progress into phase III. Topical administration may help overcome such problems. Indeed, a topical HC-030031 gel (0.05%) reversed mechanical and cold allodynia in mice after ultraviolet B-induced burn injury[90]. This implies a therapeutic value for TRPA1 antagonist creams for patients with sunburn pain or thermal injury.

Interestingly, TRPA1 inhibition produces analgesia against modalities that are not mediated by TRPA1-expressing neurons[91]. This behaviour is not unprecedented, and has been observed in other TRP receptors, such as TRPV1. Although mechanosensitive nerves do not express TRPV1, resiniferatoxin desensitizes capsaicin-sensitive afferents, resulting in ameliorated mechanical hyperalgesia (and, paradoxically, cold hyperalgesia) in a murine model of arthritic pain[92]. The molecular underpinnings of this phenomenon are not clear. One possibility is that TRPV1 expression is plastic and nerve fibres that do not express TRPV1 under normal conditions might do so under pathological conditions, such as pain[93]. Similar considerations may also apply to TRPA1.

The journey of TRPA1 antagonists as pain therapeutics to the clinic was given new impetus by the recent purchase by Eli Lilly of the Hydra Biosciences molecules[94]. As of today, TRPA1 small molecule patents have been filed by Ajinomoto Co., Algomedix Inc, Almirall S.A., Boehringer Ingelheim, EA Pharm Co., Eli Lilly, Galderma, Genentech/Roche, Glenmark, Mandom Corp and Orion Corp[95].

The 'cold and menthol receptor', TRPM8. According to a new model of sensory coding, there are two distinct neuronal populations involved in warmth perception: one population is excited by warmth, whereas the other is blocked by it[18]. This latter population expresses TRPM8 and displays ongoing cool-driven firing[18]. Accordingly, *Trpm8*$^{-/-}$ mice are incapable of distinguishing warm from cold, and show markedly attenuated cold allodynia[96,97]. Moreover, in humans, reduced *TRPM8* gene expression is associated with a reduced risk for migraine, and reduced sensitivity to cold and cold pain[39]. These observations imply a therapeutic potential for small molecule TRPM8 antagonists in the management of cold-induced pain and migraine[98].

Potent and selective TRPM8 antagonists with acceptable clinical safety profiles have been discovered[98], many of which bind competitively to the menthol or icilin binding site in the S1–S4 sensor domain (FIG. 3g). Accordingly, these antagonists demonstrated a clear-cut exposure–efficacy relationship in preclinical models, including icilin-induced wet dog shakes in rats, cold pressor test in rodents, and menthol challenge in guinea pigs; these findings predict target engagement for therapeutic dose in humans[98].

Interestingly, like TRPV1, TRPM8 displayed a basal activation tone[99]. TRPM8 antagonists evoked a mild hypothermic response, but this was not considered a hurdle to clinical use[100]. Some TRPM8 antagonists (such as AMG-333 and PF-05105679)[100,101], have already progressed into clinical studies, in which they reduced pain in the cold pressor test. Although PF-05105679 did not cause perceptible hypothermia, some volunteers reported a 'hot feeling' in the perioral area that was deemed to be non-tolerable by two study subjects[100]. AMG-333 also caused a few grade 1 adverse effects related to TRPM8 antagonism[101].

Although most of the attention was focused on antagonists, TRPM8 agonists (such as WS-12 and di-isopropyl-phosphinoyl-alkane, DIPA) also have an analgesic potential. For instance, DIPA was shown to

Cold allodynia
Paradoxical burning sensation when exposed to a cold surface.

Complex regional pain syndrome type I
Also known as reflex sympathetic dystrophy, this syndrome presents as continuous pain and sudomotor activity that is disproportionate to the initiating event.

Wet dog shakes
Rapid and alternating head rotation in rats, an animal model used to quantify central 5-HT2A activity.

Cold pressor test
Assessment of autonomic nervous system function, pain threshold and pain tolerance.

reduce spontaneous painful contractions in the human distal colon[102].

Fading and emerging TRP targets for pain relief. The classical pain targets of TRPV1, TRPA1 and TRPM8 are all expressed in nociceptors[25,40]. The expression pattern of novel TRP targets for pain relief is more diverse, ranging from sensory neurons (TRPM3) through brain nuclei involved in pain processing (TRPC4 and TRPC5) to the epithelium (TRPV3 and TRPV4) and immune cells (TRPM2).

Gain-of-function mutations in *TRPV3* were discovered in patients with Olmstedt disease[103,104], erythromelalgia[104], and painful plantar keratoderma[105]. As with TRPA1 above, many of these *TRPV3* gain-of-function mutations are at the elbow connecting the S4–S5 linker and the S5 helix (FIG. 3b). Interest in TRPV3 as an analgesic target was first raised by the observation that tissue damage upregulates TRPV3 expression in human sensory ganglia or skin[106]. Several companies developed potent and selective TRPV3 antagonists[107]. In 2014, the Sanofi-Aventis/Glenmark compound GRC15300 failed a phase II trial in patients with peripheral neuropathy, and the programme was terminated.

Olmsted disease
Also known as mutilating palmoplantar keratoderma with periorificial keratotic plaques, this is a rare congenital disorder caused by abnormal growth of the skin; it is associated with itching, pain, skin fissures and skin cancers.

Erythromelalgia
Also known as Mitchell disease, this is intense, burning pain (algia) associated with redness (erythro) that primarily affects the feet (mel).

Painful plantar keratoderma
Painful, symmetric callus formation on the pressure points of the soles.

Genetic deletion or knockdown of *Trpv4* in mice leads to attenuated pain behaviour in various preclinical models. According to these studies, TRPV4 may play a pivotal part in visceral pain[108]. An interesting approach is to use TRPA1/TRPV4 dual inhibitors for chemotherapy-associated neuropathic pain[109].

The molecular mechanisms that underlie the transition from acute to chronic neuropathic pain are largely unknown, hampering drug development. In this transition, TRPV1, TRPA1 and TRPM2 are emerging as important players[110,111]. For instance, acute pancreatitis has a large component of neurogenic inflammation that can be attenuated by TRPV1 and TRPA1 antagonists[112,113]. These antagonists also prevented the spouting of pancreatic sensory nerve fibres during the acute pancreatitis attacks, and thereby averted the development of chronic neuropathic pain. As for TRPM2, it is highly expressed in immune cells — including macrophages and microglia — that contribute to inflammatory and neuropathic pain[114]. In a murine model of neuropathic pain, *Trpm2* knockdown prevented the development of pain-related behaviour following chronic constriction injury[114].

TRPM3 has a central role in noxious heat sensing[115], making the 'triad of TRPV1/TRPA1/TRPM3' a potential multireceptor pain target[116]. The TRPM3 agonist CIM0216 induced heat hypersensitivity in wild-type mice but not in *Trpm3*$^{-/-}$ mice[117]. Conversely, TRPM3 antagonists reduced pain responses in several protocols in preclinical studies[118–120]; again, these results were validated in *Trpm3*$^{-/-}$ animals[117].

TRPC4 and TRPC5 are non-selective cation channels that can form homomers and heteromers and are expressed mostly in the amygdala and hippocampus (FIG. 4), but also in peripheral sensory neurons both in rodents[121] and humans[122]. Studies on *Trpc4*$^{-/-}$ rats showed tolerance to visceral pain responses, whereas somatic pain responses were unaffected[123]. Furthermore, the non-selective TRPC4/TRPC5 antagonist 4-methyl-2-(1-piperidinyl)quinoline (ML-204) inhibited visceral pain responses in wild-type rats[124], confirming the role of TRPC4 in visceral pain. Local application of ML-204 to amygdala attenuated neuropathic pain behaviour in rats with spared nerve injury[124]. Genetic inactivation (*Trpc5*$^{-/-}$ mice) or pharmacological blockade of TRPC5 by the small molecule antagonist AC1903 prevented the development of mechanical hypersensitivity and persistent spontaneous or tactile pain in a wide range of murine pain models, including that induced by skin incision, chemotherapy and complete Freund adjuvant injection[122]. We note that these pain conditions are associated with elevated lysophosphatidylcholine (LPC) levels, and exogenous LPS triggers TRPC5-dependent pain behaviour in naive mice[122]; LPS also causes intensive itching during cholestasis both in rodents and nonhuman primates by activating TRPV4 in skin keratinocytes[125]. Because TRPC5 also regulates prolactin release[126], TRPC5 antagonists may have enhanced analgesic efficacy in females given that prolactin promotes pain only in that sex. These findings suggest that centrally acting TRPC4 and TRPC5 antagonists could relieve visceral and neuropathic pain, or at least make it more tolerable[127].

Respiratory disease

TRP channels are attractive targets for the treatment of respiratory diseases[128] given their widespread expression in the lung, both in immune and structural cells, and their central role in evoking respiratory symptoms like bronchospasm and cough.

TRPV1 in respiratory disease. Inhaled capsaicin evokes coughing in both guinea pigs and humans by activating TRPV1 on C-fibre afferents[129]. Indeed, capsaicin-containing sprays and gas canisters are used by individuals for personal protection and by law enforcement for crowd control[16]. Increased cough reflex sensitivity to inhaled capsaicin has been observed in patients with asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and chronic idiopathic cough. These findings suggested that TRPV1 is a credible target to treat both chronic idiopathic cough and cough from inflammatory lung disease[130]. The increased TRPV1 activity may be explained by elevated levels of inflammatory mediators (such as prostaglandin E_2, neurotrophins and bradykinin) in the airways of patients with asthma and with COPD. These endogenous substances activate airway sensory nerves, causing coughing[131]. Moreover, neurons undergo phenotypical changes in respiratory diseases both in experimental animals and human challenge models. For instance, in guinea pigs and rats challenged with ovalbumin, or in guinea pigs treated with neurotrophins, the number of TRPV1-positive neurons (particularly of the nodose-originating Aδ subtype) increases, suggesting that changes in neural pathways influenced by their local environment may result in exaggerated functional responses[132,133]. Moreover, *TRPV1* single nucleotide polymorphisms (SNPs) have been associated with coughing, suggesting that *TRPV1* variants may enhance susceptibility to coughing in smokers and in subjects with a history of workplace exposure[134].

Clinical studies tested the postulated causal role of TRPV1 in patients with chronic idiopathic cough and in chronic cough associated with COPD in patient groups with increased cough sensitivity to capsaicin. In patients with chronic idiopathic cough, the TRPV1 antagonist SB-705498 caused a small but statistically significant inhibition of capsaicin-evoked coughing, but no effect on spontaneous coughing frequency[135]. In subsequent studies, XEN-D0501 (a TRPV1 antagonist with superior efficacy and potency) did not improve spontaneous cough frequency in patients with refractory coughing[136], nor did it affect spontaneous coughing in patients with COPD[137], which effectively rules out TRPV1 as a relevant therapeutic target for chronic cough in these patient groups, but not in other respiratory diseases associated with exaggerated coughing.

There is widespread non-neuronal TRPV1 expression in the lung, in both structural (such as fibroblast) and immune cells (such as alveolar macrophages)[138]. For example, TRPV1 was detected in human bronchial epithelial cells, with increased expression in patients with refractory asthma[139]. In bronchial epithelium, TRPV1 activation mediates mucin secretion induced by acid and particules[140], as well as the release of cytokines[141] and ATP[142]. In a preclinical model of cigarette smoke exposure used to mimic the inflammatory response seen in COPD, the TRPV1 inhibitor JNJ17203212 reduced cigarette smoke-induced ATP release from human bronchial epithelial cells[142]. Furthermore, bronchiolar lavage fluid collected from *Trpv1*$^{-/-}$ mice following cigarette smoke exposure had diminished ATP release and neutrophilia compared with that of wild-type mice[142]. Conversely, whole-lung homogenates from patients with COPD showed increased *TRPV1* mRNA expression compared with samples from smokers without COPD and healthy non-smokers, suggesting an association between TRPV1 expression and disease pathophysiology[142].

The role of TRPV1 in asthma is controversial; some studies show no impact whereas others show protection through TRPV1 inhibition by antagonists or genetic inactivation. For instance, in rodent ovalbumin challenge models, TRPV1 blockade improved standard end points, including eosinophilia, airway hyperresponsiveness and the late asthmatic response[143]. Furthermore, TRPV1 inhibition reduced the interleukin (IL)-13-driven asthma phenotype in mice, and blocked airway hyperresponsiveness to histamine in guinea pigs following ovalbumin exposure[144]. Similarly, data from a murine house-dust mite model suggested that TRPV1, but not TRPA1, inhibition reduced airway cellular inflammation and airway hyperresponsiveness[143]. Although many of the effects seen in this study were not statistically significant, there was a consistent suppression across the functional end points measured, suggesting a role for TRPV1 in CD4$^+$-dependent allergic asthma models.

In summary, the reasons for the observed differences in studies investigating a role for TRPV1 in allergic asthma are unclear, but could be due to differences in species, strains, antigens and interventions used to dissect TRPV1 biology.

TRPA1 antagonists. TRPV1 and TRPA1 are often coexpressed in sensory neurons that innervate the airways, but they are also found separately[128,138,145]. TRPA1 agonists (like cinnamaldehyde) evoke human vagus nerve depolarization and induce coughing in both guinea pigs and human volunteers[146]. Conversely, TRPA1 antagonists (such as GRC-17536 and HC-030031) are potential anti-tussive agents[147,148]. In the lung, TRPA1 is also expressed in bronchial epithelial cells and fibroblasts[149].

TRPA1 is an interesting respiratory target because it is activated by known lung irritants including natural products, such as allyl isothiocyanate, allicin and cannabinol[150], found in mustard oil, garlic and cannabis, and by environmental irritants, such as acrolein[151], that are present in air pollution and cigarette smoke[152]. These electrophilic agonists covalently attach to a hypersensitive cysteine in the cytoplasmic domain of TRPA1 (REF.[82]) (FIG. 3c). TRPA1 is also activated by reactive and electrophilic by-products of oxidative stress (such as ROS), and electrophiles like hypochlorite and hydrogen peroxide[81,153]. Diesel exhaust particles can also activate airway C-fibre afferents via TRPA1 (REF.[154]). These particles contain polycyclic aromatic hydrocarbons that stimulate mitochondrial ROS production by interacting at the aryl hydrocarbon receptor. This observation links diesel exposure to respiratory symptoms[154].

In addition to environmental irritants, TRPA1 is also activated indirectly by inflammatory mediators[151] (such as bradykinin, PGE_2 and prostaglandin D_2), which are elevated in the bronchoalveolar lavage fluid of patients with asthma and COPD[155]. We note that the exhaled breath of patients with inflammatory airway disease is more acidic than that of healthy volunteers[156]. This is interesting because inhalation of low pH solutions, such as citric acid, causes coughing in both guinea pigs and humans. In fact, inhaled citric acid is used in the clinic to evaluate cough reflex sensitivity. Citric acid was thought to evoke cough by virtue of its low pH and subsequent activation of TRPV1 and acid-sensing ion channels (ASIC)[157,158]. However, TRPA1 antagonists (such as GRC-17536) also inhibited citric acid-induced coughing in conscious guinea pig models[147]. It is not yet known whether the low pH component of citric acid or the resulting increased osmolarity is responsible for activating TRPA1 (or TRPV1) to evoke coughing.

TRPA1 has been implicated in the pathophysiology of allergic asthma[159,160]. In allergic individuals, exposure to relevant antigens can lead to an early asthmatic response followed, in certain patients, by a corticosteroid-sensitive, late asthmatic response. Despite its widespread use in the clinical assessment of new therapeutic entities, the mechanisms underlying late asthmatic response remain unclear. Following allergen challenge, activation of TRPA1 stimulates vagal broncho-pulmonary C-fibres and this induces late asthmatic response in the Brown Norway rat asthma model[159]. The mechanism of action is unclear, but the allergen probably stimulates the channel indirectly via the release of endogenous TRPA1 activators, possibly mast cell products like tryptase.

TRPA1 may also have a key role in the airway hyperresponsiveness characteristic of asthma. Indeed, the

TRPA1 antagonist, HC-030031, reverses airway hyperresponsiveness induced by acetylcholine in an ovalbumin mouse model[159]. Toluene di-isocyanate (TDI), a reactive compound used in the manufacture of polymeric derivatives and known to activate TRPA1 (REF.[161]), can also evoke respiratory symptoms, including late asthmatic response, in exposed workers[162]. Non-allergic airway hyperresponsiveness can be induced by a single exposure of hypochlorite (a known TRPA1 agonist) in ovalbumin-exposed wild-type mice but not in *Trpa1*$^{-/-}$ mice[163]. Ovalbumin-challenged wild-type rats showed signs of airway inflammation[86], whereas *Trpa1*$^{-/-}$ rats did not, which again suggests that TRPA1 inhibition has therapeutic potential for asthma.

Lower respiratory tract infections are a leading cause of death in adults and pneumonia is the single largest cause of death in children[164]. Coughing is the main method of spreading bacteria from a human host into the environment. However, the mechanisms of coughing in patients with lower respiratory tract infections are unknown. We note that lipopolysaccharide, which is found in the outer membrane of Gram-negative bacteria, has been identified as a TRPA1 activator that exerts fast excitatory actions via TRPA1 independent of Toll-like receptor-4 (TLR4) activation[165]. This finding implicates TRPA1 as a driver of respiratory symptoms following bacterial infections and warrants further investigation.

Although associations have been found between *TRPA1* SNPs and susceptibility to airway disease[166], the evidence linking TRPA1 dysfunction to respiratory disease pathophysiology is still rudimentary. Drug discovery efforts have been hampered by the limited bioavailability of TRPA1 antagonists[167,168]. Recently, a potent, selective and orally bioavailable small molecule TRPA1 antagonist, GDC-0334, with good target engagement in human volunteers, has been reported[169]. In preclinical models of respiratory disease, GDC-0334 inhibited cough response, airway smooth muscle hyperreactivity and oedema formation in several species[169]. It is hoped that clinical studies with GDC-0332 (or other compounds with good bioavailability) will answer the question of whether blocking TRPA1 in respiratory disorders has therapeutic promise[168].

Box 2 | Potential indications for TRP channel ligands: COVID-19 and cognitive decline in diabetes

SARS-CoV-2 may cause potentially fatal acute respiratory syndrome (ARDS) and pulmonary oedema in a subset of patients with COVID-19. As several TRPV4 antagonists that have already been proved safe in clinical trials can protect the alveolo-capillary barrier, these compounds could be protective in patients with COVID-19 at risk of lung oedema[179]. The afferent TRPV1-expressing pulmonary innervation has also been implicated in ARDS; drugs that silence these fibres (for example, resiniferatoxin) can also improve clinical outcome[297]. Deadly viruses can exploit the endo-lysosomal trafficking system of the host cells for penetration and replication. The integrity of this system depends on mucolipins (TRPMLs). Therefore, compounds that interfere with endo-lysosomal maturation and trafficking via TRPMLs may prevent the virus from entering the host cells[298].

In the brain, blood flow must meet the dynamically changing metabolic demands of active neuronal populations. The machinery of neuro-vascular coupling (NVC) senses the change in neuronal activity and redirects the blood flow accordingly. Most recently, TRPA1 expressed in capillary endothelium has emerged as a key player in this functional hyperaemic response[299]. In response to neuronal activity, TRPA1 initiates a rapid Ca^{2+} signal, which is dependent on the endothelial pannexin-1 channel and purinergic P2X receptors[299]. This current is subsequently converted into an inward rectifying K^+-mediated electric signal that guides blood flow by regulating the tone of the arteriole wall. Disturbed NVC is thought to be a major risk factor for cognitive impairment in diabetic patients[300]. In diabetes, methylglyoxal (and probably also other harmful by-products of glycolysis) bind to and activate TRPA1 (REF.[79]). Therefore, one can speculate that pathogenic metabolic products may impair NVC in diabetic patients in a capillary endothelial TRPA1-dependent fashion. If so, TRPA1 antagonists could prevent cognitive decline by protecting the NVC in patients with diabetes in addition to protecting nerve fibres[88] and relieving pain during diabetic polyneuropathy[87]. This would be an important added benefit of TRPA1 antagonist therapy.

TRPV4 antagonists. TRPV4 is expressed in a wide range of non-neuronal cell types in human airways, including airway and vascular smooth muscle, epithelial cells, fibroblasts and inflammatory cells (such as macrophages and neutrophils)[4]. Multiple endogenous pro-inflammatory and environmental stimuli act in concert to activate TRPV4 (REF.[4]). Accordingly, TRPV4 activation is implicated in the pathophysiology of chronic lung diseases[170–172].

TRPV4 was referred to as 'the gatekeeper of pulmonary capillary permeability'. Its contribution to respiratory disease is probably best understood in the development of acute lung injury[173]. TRPV4 is involved in disrupting the epithelial and endothelial barrier function[173], which suggests an important role in lung oedema formation associated with inflammation and tissue injury[174]. In the rodent lung, TRPV4 blockade, or knockdown, inhibited ventilator- and acid-induced lung injury, by significantly reducing the infiltration of inflammatory cells (including neutrophils and macrophages) and lung injury scores[175,176]. These data suggest a broad-spectrum inhibition of acute lung injury regardless of causality. Indeed, TRPV4 inhibitors are developed by the National Institute of Health (NIH) as countermeasures against chemical threats[177].

High pulmonary venous pressure is a major cause of heart failure. TRPV4 antagonists inhibit pulmonary oedema associated with heart failure in animal models[178], paving the way towards clinical trials. On the basis of these observations, calls have been made to evaluate the effect of TRPV4 inhibitors in COVID-19 patients at risk of lung oedema[179] (BOX 2).

TRPV4 is also implicated in chronic respiratory diseases such as asthma, COPD and chronic refractory cough[170–172]. Its role in diverse pathologies across respiratory disease has been explained by TRPV4-induced ATP release in a pannexin 1-dependent manner with resultant activation of purinoceptors[180,181]. Consistent with this hypothesis, TRPV4-induced ATP release was demonstrated in human airway epithelial cells[142,182] and smooth muscle cells[181]. TRPV4 and purinoreceptor P2X3 antagonists were shown to inhibit Aδ sensory afferent nerve fibre activation and coughing induced by TRPV4 agonists or hypo-osmolar solutions (known to activate TRPV4)[183]. This study identified the TRPV4–ATP–P2X3 axis as a driver of airway sensory nerve reflexes such as coughing, and clinical trials with P2X3 receptor antagonists, such as AF-219, would support the role of ATP as a driver of the cough reflex[183].

In the context of asthma, TRPV4 agonists can induce a mast cell-dependent, contractile response in human

SMAD
Downstream signal transducer for the receptors of the transforming growth factor β (TGFβ) superfamily.

Neurogenic bladder
Urinary condition caused by impaired neuronal control (for example, by spinal cord injury or multiple sclerosis) of bladder function.

Interstitial cystitis
Poorly understood clinical condition that predominantly affects young women and causes recurrent pain in the pelvic region associated with problems with urination.

Akita mice
Genetic mouse model of type 1 diabetes.

bronchial and guinea pig tracheal airway smooth muscle in vitro. This effect is attributed to TRPV4-induced ATP release from airway smooth muscle[181], which activates P2X4 receptors on mast cells to release cysteinyl leukotrienes (Cys LT); this, in turn, activates the Cys LT-1 receptor to evoke contraction[184].

COPD is an inflammatory lung disease associated with cigarette smoking. Smoke exposure was reported to cause a dose-dependent increase in ATP release — which may drive the airway inflammation — from primary human bronchial epithelial cells, and this effect was attenuated by blockers of TRPV1, TRPV4 and pannexin-1 channels[142]. Interestingly, lung tissue from patients with COPD shows an increase in *TRPV4* mRNA expression compared with healthy smokers and non-smokers[142].

Genetic variants of *TRPV4* have been associated with asthma[185] and COPD[186]. For example, the loss-of-function variant, TRPV4-P19S, was linked to childhood asthma[185], and a genome-wide association study highlighted seven *TRPV4* SNPs that conferred increased susceptibility to COPD[186]. It was postulated that TRPV4 modulators could influence COPD pathophysiology, but this hypothesis has yet to be tested.

TRPV4 activity is upregulated in lung fibroblasts derived from patients with IPF[187]. In IPF models, *Trpv4*$^{-/-}$ mice are protected from fibrosis[187]. TRPV4 modulates transforming growth factor (TGF)-β1-dependent actions in a SMAD-independent manner with enhanced actomyosin remodelling and increased nuclear translocation of the α-smooth muscle actin-transcription co-activator, myocardin-related transcription factor (MRTF)-A[188,189]. These data point to TRPV4 inhibition as a potential therapeutic approach in pulmonary fibrosis[189]. Interestingly, like asthma and COPD, increased amounts of ATP have been detected in the airways of patients with IPF[190], but currently there is no evidence linking the TRPV4/pannexin/ATP axis to IPF.

TRPM8. TRPM8 is a cold-responsive receptor[7,8] that, in principle, may mediate coughing and bronchoconstriction caused by inhalation of cold air. The role of TRPM8 activation in cough is, however, controversial because menthol, a TRPM8 agonist, paradoxically inhibits citric acid-induced cough in both guinea pigs[191] and humans[192], and causes a temporary decrease in capsaicin-evoked cough in healthy individuals[193]. Indeed, menthol inhibits sensory nerve activation and is used as an over-the-counter antitussive medication. Menthol was — and in many countries still is — often added to cigarettes to inhibit irritancy in the airways, which may promote nicotine addiction. Therefore, menthol is now banned from cigarettes in the USA.

There is still a big question mark over whether the beneficial effects of menthol in the lung are mediated via activation of TRPM8. This is likely to be confounded by the lack of selective tools. For example, in addition to their effects on TRPM8, both menthol and icilin activate and then block TRPA1 (REF.[194]) (FIG. 3c,d,g). Several recently developed TRPM8 inhibitors should help to elucidate the role of this channel in respiratory disease biology and pathophysiology.

The genito-urinary system

In the neurogenic bladder, the TRPV1-expressing C fibre-driven micturition reflex, which is inactive under physiological conditions, resumes control of the bladder functions[16]. Intravesical administration of a large enough dose of TRPV1 agonist (capsaicin[195] or resiniferatoxin[196]) to desensitize the C-fibres provides lasting relief in patients with neurogenic bladder by increasing bladder capacity and reducing the number of incontinent episodes[197]. Furthermore, in bladder biopsy samples taken from patients with interstitial cystitis, the density of TRPV1-expressing fibres correlated with clinical symptoms[198]. Yet, in a phase II clinical trial intravesical resiniferatoxin failed to meet the primary end point (symptom improvement according to the Global Response Assessment, a 7-point scale rating overall change in symptoms), faring no better than placebo after 4 weeks[199]. Therefore, it is likely that the increased TRPV1 expression in the bladder biopsy samples was not the cause but a consequence of the disease. Parenthetically, an interesting (and controversial) indication of topical resiniferatoxin desensitization is its application to the penis to prevent premature ejaculation[200].

Animal experiments suggested a therapeutic value for TRPV1 (GRC-6211)[201] and TRPM8 (RQ-00434739 (REF.[202]) and KRP-2529 (REF.[203])) antagonists for suppressing hyperactivity in the chronically inflamed bladder, which is yet to be tested in the clinic.

In the bladder, TRPV4 is expressed both in urothelium and in the detrusor muscle, where its activation causes sustained muscle contractions[204]. This is consistent with the spotty incontinence phenotype of the *Trpv4*$^{-/-}$ mice[205]. By contrast, the TRPV4 agonist GSK1016790A evokes bladder contractions[206]. Of relevance, TRPV4 expression is increased in human overactive bladder mucosa[207]. These and other preclinical findings imply that a TRPV4 agonist may improve the underactive bladder[208], whereas an antagonist could be valuable in managing the overactive bladder[209].

The TRPC5 antagonist AC-1903 prevented podocyte loss in a rodent model of focal segmental glomerulosclerosis, suggesting a novel interaction for renal allograft protection[210]. TRPC6 is highly expressed in the kidney, but the role of TRPC6 in renal pathology is complex, which has hindered drug development. For example, BI-749327, a selective TRPC6 antagonist, ameliorates renal fibrosis and dysfunction[211], whereas deletion of *Trpc6* worsens glomerular injury in Akita mice[212]. Accordingly, a loss-of-function *TRPC6* variant (G757D) is associated with focal segmental glomerulosclerosis[213]. These latter observations warrant caution when using TRPC6 blockers in humans.

Dermatology and ophthalmology

In the skin and the eye, TRPs are attractive pharmacological targets because they are amenable to topical therapy, probably reducing the risk of serious adverse effects. TRP channels are broadly expressed in various skin cell types (including keratinocytes, melanocytes, skin appendage cells, nerve endings and immune cells), with functions ranging from skin barrier function and hair growth through wound healing to cutaneous inflammation and itching[19,78,214].

TRPA1, TRPV3, TRPV4 and, to a lesser degree, TRPV1 and TRPM8 are all promising targets to relieve itching[19]. TRPA1 expression is elevated in human psoriatic skin biopsies[215]. Accordingly, *Trpa1*$^{-/-}$ mice show reduced scratching behaviour in a murine model of chronic itching, and lack the extensive epidermal hyperplasia prevalent in psoriasis and atopic dermatitis[215]. Transgenic mice with skin-targeted, gain-of-function *Trpv3*^{Gly573Ser} (FIG. 3b) exhibit scratching behaviour[216]. In humans, gain-of-function *TRPV3* mutations (G568D and Q580P; FIG. 3b) were described in palmoplantar keratoderma[105], and increased TRPV3 expression was reported in post-burn pruritus[217]. Similarly, TRPV4 is overexpressed in skin biopsy samples from patients with chronic pruritus[218], and genetic deletion of *Trpv4* ameliorates itching in mouse models of chronic itch[219]. A TRPM8 agonist (menthoxypropanediol) cream was also reported to relieve human itching[220].

The TRPV1 antagonist PAC-14028 (now in phase III clinical trials) improves skin barrier function and relieves pruritus in patients with atopic dermatitis[221]. Although topical TRPV1 antagonists are generally regarded as harmless, a recent study suggests caution. In TRPV1-Ai32 optogenic mice, cutaneous light stimulation activated TRPV1-expressing neurons with resultant local-type 17 immune response[222], in which a cascade of cytokine-mediated events leads to activation of antimicrobial responses in keratinocytes and recruitment of neutrophils to the skin. If this observation holds true in humans, patients treated with topical TRPV1 antagonists may be susceptible to cutaneous fungal and bacterial infections.

TRPA1 is also involved in ultraviolet radiation-induced burn. In mice, topical administration of the TRPA1 antagonist, HC-030031, applied after irradiation blocked the development of mechanical and thermal allodynia[223].

TRPV1 and TRPV3 have been implicated in hair growth. For example, *Trpv3* null mice have a thick, wavy coat of hair[224] whereas mice with constitutively active *Trpv3* (the DS-*Nh* strain) are hairless[225]. Indeed, TRPV3 activation has been shown to inhibit human hair growth[226]. Accordingly, topical TRPV3 agonists and antagonists may be beneficial in patients with hirsutism and alopecia, respectively. Nude DS-*Nh* animals also develop a skin condition similar to human atopic dermatitis[227]. Furthermore, TRPV3 activation blocks lipogenesis in human sebocytes[228], suggesting a therapeutic potential for a TRPV3 antagonist in dry skin dermatoses.

TRPV1 has been implicated in psoriasis[229] and TRPV4 was named as a potential target in rosacea[230]. Recently, gain-of-function *TRPM4* mutations (I1033M and I1040T; FIG. 3d) were linked to erythrokeratodermia[231]. Even if their connection to TRP channels is still weak, all these diseases represent unmet medical needs.

Eye drops containing a TRPM8 agonist help to moisturize the cornea in patients with dry eye disease[232]. In animal experiments, the TRPV4 antagonist, HC-067047, protected against the fibrosis (stromal opacification) that develops following alkali burn injury[233]. Last, intraocular TRPV1 antagonist administration has been proposed to relieve allergic conjunctivitis[234].

Optogenic mice
Genetically modified mice expressing light-sensitive ion channels in neurons; light is used to control neuronal function in vivo.

Psoriasis
Disease of the skin that causes red, flaky, crusty plaques.

Rosacea
A common skin condition that causes redness and visible blood vessels in the face; it may also affect the nose (rhinophyma) and the eyes (dry, irritated, swollen eyes).

Erythrokeratodermia
A group of keratinization disorders that manifest in erythema (redness) and hyperkeratosis (scaling); most cases are indolent with no effect on general health.

Multiple sclerosis
A neurological disease in which inflammation is thought to drive disease progress.

Central nervous system

TRPA1 as a therapeutic target in stroke and in multiple sclerosis. Although *Trpa1*$^{-/-}$ mice lack any obvious neurological phenotype and there is little, if any, detectable mRNA signal in the central nervous system[235], functional TRPA1 seems to be present, albeit at low levels, in the brain: in glial cells (astrocytes and oligodendrocytes)[236], in cortical neurons[237], in cerebral endothelium[238] and in Schwann cells[239]. This discrepancy between positive function-based TRPA1 results and weak or missing *Trpa1* mRNA signals is puzzling and yet to be explained.

The potential therapeutic use of brain-permeable TRPA1 antagonists for neuroprotection has emerged from an elegant electrophysiological study[240]. White matter is particularly vulnerable to hypoxia associated with stroke. Glutamate release and subsequent activation of N-methyl-D-aspartate (NMDA) receptors in oligodendrocytes is thought to underlie hypoxia-induced white matter injury[240]. Pharmacological or genetic inactivation of TRPA1 prevented hypoxia from damaging and ultimately killing oligodendrocytes after stroke[240]. On a related note, TRPV1-mediated hypothermia reduced stroke volume by 50% and promoted functional recovery after ischaemic stroke in mice[30].

Oligodendrocytes are also affected in multiple sclerosis. Cuprizone-induced demyelination is widely used as a multiple sclerosis model to assess the potential therapeutic efficacy of novel multiple sclerosis treatments. In this model, pharmacological blockade or genetic deletion of *TRPA1* reduced oligodendrocyte apoptosis[241]. Furthermore, methylglyoxal, a known TRPA1 activator[79], accumulated in the white matter lesions of multiple sclerosis[242]. Dimethyl fumarate, used to treat multiple sclerosis and psoriasis, and known to activate the antioxidant response through Nuclear factor erythroid 2-related factor 2 (NRF2), was found to activate TRPA1 in immune cells independent of NRF2 (REF.[243]). This novel NRF2-independent mechanism may contribute to the peripherally restricted immunosuppressive action of dimethyl fumarate.

TRPA1 in anxiety, dementia and seizures. *Trpa1*$^{-/-}$ mice show reduced anxiety-like behaviour, and this beneficial effect can be reproduced with TRPA1 antagonists in healthy, wild-type mice[244]. The neural circuit responsible for the antidepressant action of TRPA1 antagonism is currently unknown. Importantly, ageing *Trpa1*$^{-/-}$ mice also exhibited improved memory (fewer reference memory errors), implying a role for TRPA1 in age-related memory decline[245]. Together, these findings identify TRPA1 as a potential target in the pharmacotherapy of anxiety and dementia, a common combination in the elderly (for TRPA1 in capillary endothelium in the brain, and memory decline in diabetic patients, see BOX 2).

However, *Trpa1*$^{-/-}$ mice also show defects in white matter myelination, with the myelin basic protein level downregulated and the number of mature oligodendrocytes reduced[246]. According to this observation, TRPA1 may play a critical part in the maturation process of

Box 3 | Phenome-wide association studies to support TRP channel target validation

The complex role of TRP channels in various physiological functions poses the question of whether the risk–benefit ratio of a given TRP channel is attractive enough to start a drug discovery programme that may deliver novel drug candidates after only 5–10 years of hard work. Another issue related to drug target validation is how well efficacy and safety findings in animal models translate to human studies. It is generally accepted that incorporating human-relevant data at early stages of the drug discovery process increases the likelihood of success. A recent exciting development to accelerate drug target validation is to use large-scale, real-world patient cohorts from biobanks and other resources to associate phenotypes with genotypes in an unbiased manner[301]. Indeed, there is evidence that targets with genetic support are approximately twice as likely to lead to an approved drug compared with those without such support. A genetic variant of a given protein may be associated with a particular disease-relevant phenotype without obvious safety implications or, vice versa, a variant may be implicated in a given disease along with several additional unwanted phenotypes. The recent availability of genetic epidemiological tools such as phenome-wide association studies[301] and Mendelian randomization, as well as large-scale real-world clinical datasets, allows for the reliable prediction of in vivo consequences of the modulation of a given target directly in humans. Efficacy and safety signals derived from such 'virtual' trials are increasingly likely to drive the benefit–risk discussions for individual TRP channels.

oligodendrocytes, because the myelination process is likely to continue throughout life during new skill learning, and therefore chronic and complete block of TRPA1 in oligodendrocytes may interfere with myelination. However, robust therapeutic efficacy can probably be achieved during intermittent partial pharmacological block of TRPA1 in humans, allowing preservation of the physiological house-keeping function of TRPA1.

TRPA1 in astrocytes may constitutively regulate excitatory neurotransmission in healthy brain[237]. If confirmed, central TRPA1 inhibition may interfere with memory formation, contradicting the memory phenotype observed in *Trpa1*$^{-/-}$ mice[245]. By contrast, a phase I study of a centrally acting TRPA1 antagonist in healthy volunteers revealed no central nervous system toxicity issues at concentrations that were several-fold above the IC$_{50}$ value[247]. One potential explanation for this discrepancy is that constitutive TRPA1 activation in brain slice astrocytes is an experimental artefact: large amounts of reactive lipid hydroperoxides and other putative TRPA1 agonists may be leaking from damaged and dead cells.

Hippocampal slices from an Alzheimer disease (AD) mouse model provided compelling evidence that TRPA1 activation by β amyloid contributes to network hyperexcitability, possibly driving silent seizures[248]. This is important because antiepileptic drug use in AD is associated with increased risk for stroke[249]. Central TRPA1 antagonism may provide a safe therapy for seizures in AD.

Parenthetically, in the triple-transgenic AD mouse model (3xTg-AD$^{+/+}$), *Trpv1*$^{-/-}$ animals had better memory function and lower tau accumulation in the hippocampus compared with *Trpv1* wild-type mice[250]. The challenge, of course, is to deliver TRPA1 and/or TRPV1 antagonists to the central nervous system without causing unacceptable systemic side effects.

TRPM2, TRPM3 and TRPM4: from stroke to bipolar disorder. TRPM2 is a well established redox sensor[251] and a potential target in hypoxic-ischaemic brain injury[252]. TRPM2 is implicated in neuronal death by ROS[251,252]. Indeed, the TRPM2 antagonist JNJ-28583113 protects cells from oxidative stress-induced death[253]. In a mouse model of cardiac arrest and cardiopulmonary resuscitation, TRPM2 inhibition improved functional recovery following cerebral ischaemia[254]. In the hippocampus of patients with major depressive disorder, increased *TRPM2* mRNA levels were detected, suggesting TRPM2 as a target for treating depression[255]. *TRPM2* is also a susceptibility gene for bipolar disorder[256]. *Trpm2*$^{-/-}$ mice display impaired social cognition, and a subset of bipolar patients possess the hypoactive D543E *TRPM2* gene variant[256] (FIG. 3d). The connection between TRPM2 and bipolar disorder was further strengthened by the finding that TRPM2 regulates the phosphorylation of glycogen synthase kinase-3 (GSK3), the main target of lithium[257].

In the central nervous system, TRPM3 is expressed in both glia and neurons. The discovery that primidone — a drug that has long been approved for the treatment of epilepsy and essential tremor — blocks TRPM3 at concentrations that are achieved in the brain during pharmacotherapy validates TRPM3 as a promising neurological drug target[258]. One may argue that excessive TRPM3 activation can drive white matter injury and thus a centrally acting TRPM3 antagonist may be disease-modifying by protecting white matter. Most recently, gain-of-function *TRPM3* variants (such as V837M; FIG. 3d) have been linked to intellectual disability and epilepsy[259], although it is not clear how this observation can be exploited for pharmacotherapy.

TRPM4 is a Ca^{2+}-activated non-selective cation channel in vascular smooth muscle that has a key role in myogenic constriction of cerebral arteries[260]. TRPM4 is closely associated with the sulfonylurea receptor-1 (SUR1)[261], which explains the sensitivity of TRPM4 to the antidiabetic compound glibenclamide, a SUR1 inhibitor. In rodent models, spinal cord injury upregulated TRPM4 during secondary haemorrhage, and the increase in haemorrhage was prevented by genetic silencing of *Trpm4* by antisense in rats or in *Trpm4*$^{-/-}$ mice[262]. In rats, siRNA-mediated silencing of *Trpm4* reduced infarct volume in a permanent stroke model, indicating that TRPM4 inhibition may improve blood–brain barrier integrity after ischaemic stroke reperfusion[263]. SUR1-TRPM4 forms a heteromer complex with aquaporin-4, which was shown to amplify ion–water osmotic coupling and thereby drive astrocyte swelling in stroke. These observations motivated investigation of the clinically well tolerated glibenclamide as a SUR1-TRPM4 antagonist in stroke patients[264]. In a small-scale study, glibenclamide was shown to improve outcome[234]. A phase III clinical trial with intravenous glibenclamide (BIIB093) is currently recruiting stroke patients (NCT02864953)[265].

TRPM4 was also shown to mediate axonal and neuronal degeneration in experimental autoimmune encephalomyelitis and such effects could be antagonized by glibenclamide, suggesting that TRPM4 inhibition may be a novel target for multiple sclerosis[266].

Inhibition of TRPCs has anxiolytic and antidepressant effects. Behavioural studies with *Trpc4*$^{-/-}$ and *Trpc5*$^{-/-}$ mice showed positive results in models that predict

Phenome-wide association study
A study designed to test for associations between a specific genetic variant (such as single nucleotide polymorphism, SNP) and a wide range of phenotypes or disease risks in a large cohort of individuals.

Lithium
Mainstay pharmacotherapy for bipolar disorder for acute mood episodes, switch prophylaxis and suicide prevention.

Lysozomes
Intracellular organelles with a key role in cellular waste handling and recycling.

Mucolipidosis type 4
An autosomal recessive lysosomal storage disorder causing delayed mental and motor development and vision impairment that worsens with time; patients present with intellectual disability (absent speech), difficulty swallowing and weak muscle tone.

Non-invasive, low-intensity, low-frequency ultrasound
(LILFU). A promising transcranial approach to stimulating brain pathways.

anxiolytic and antidepressant action[267,268]. This effect could be replicated by a small molecule, a TRPC1/TRPC4/TRPC5 pan-inhibitor, HC-070, which implies a therapeutic potential to treat anxiety disorders[269]. Hydra Biosciences and Boehringer Ingelheim have started a phase I trial with their TRPC4/5 inhibitor[270]. The *Trpc4*$^{-/-}$ rats also displayed reduced cocaine self-administration without deficits in learning for natural rewards[271]. If this observation is confirmed in humans, a TRPC4 antagonist may prove clinically useful in addiction. TRPC5 has also been implicated in oxidative neuronal death, implying a role in neuroprotection in neurodegenerative diseases like Huntington disease[272]. The recent availability of potent, selective TRPC5 antagonists like NU-6027 and AC-1903 may help test this hypothesis.

TRPML1 agonists for the treatment of neurodegenerative diseases. TRPML1 is a Ca^{2+}-permeable non-selective cation channel expressed in lysosomes[273]. Loss-of-function *TRPML1* mutations cause type-IV mucolipidosis[274], a rare genetic disease with predominantly neurological symptoms. Lysosomal Ca^{2+} signalling by TRPML1 regulates autophagy and lysosomal biogenesis[275], and TRPML1 levels in lysosomes are controlled by the transcription factor-EB (TFEB)[276]. The subcellular localization and activity of TFEB is also regulated by phosphorylation mediated by Mammalian Target of Rapamycin (mTOR)[275]. The dephosphorylated form of TFEB translocates into the nucleus, where it induces the transcription of target genes, including *TRPML1* (REF.[275]). TFEB has attracted recent attention for its ability to clear pathogenic molecules in mouse models of Parkinson and Alzheimer disease[277]. In Parkinson disease mouse models, TRPML1 regulates α-synuclein exocytosis in dopaminergic neurons[278]. On the basis of these observations, a small molecule TRPML1 agonist could be a disease-modifying agent in neurodegenerative diseases. A recent structure of TRPML1 with the agonist ML-SA1 bound under the pore helix[279] — analogous to propofol in TRPA1 or cannabidiol (CBD) in TRPV2 — could aid in developing such agonists (FIG. 3e,f). We note that Merck purchased Calporta, a company developing TRPML1 agonists for US$576 million in November 2019 (REF.[280]).

Box 4 | **Outstanding questions in the TRP field**

- Is it possible to achieve further improvement in ‘drug-likeness’ of TRPA1 antagonists: that is, improved solubility and metabolic stability while maintaining high potency?
- Only a few therapeutic TRP channel selective antibodies have so far been developed. Will more effort be put into developing therapeutic TRP channel antibodies in the future? Will novel technologies such as cryo-EM reveal high-affinity antibody binding sites?
- Would alternative treatment modalities, such as antibodies, siRNA or gene therapies, offer any advantages over small molecules?
- What are the roles of TRP channel splice variants under various disease conditions? Can cell compartment and or tissue-specific splice variants be therapeutically targeted?
- Is it possible to pharmacologically modulate intracellular TRP channels to treat chronic diseases such as lysosomal storage disease, neurodegenerative disease or intracellular pathogens through modulation of lysosomal TRP channels?
- Most TRP channel modulators are negative allosteric modulators (NAMs). We need to understand how a NAM interacts with the TRP channel activated by natural agonist in a given disease to build a quantitative PK-PD model of drug action in vivo. Do we understand well enough which endogenous agonists drive the disease process?
- TRPA1 in astrocytes was recently shown to be modulated by non-invasive, low-intensity, low-frequency ultrasound (LILFU)[302]. Will LILFU open up new avenues for non-pharmacological modulation of a disease[303]? Could LILFU improve focused delivery of big molecules to the brain through the blood–brain barrier in a safe way?
- Can we use drugs that selectively activate (or block) phosphorylated (or otherwise post-translationally modified) TRP proteins? If so, can such selectivity mitigate adverse effects?
- Using our understanding of TRP channel structures[304], can we design drugs that selectively target distinct functional states of TRP channels?

Cancer, obesity and diabetes

Several TRP channels show altered expression in cancers, but it is unclear whether this is cause or consequence of the disease. The use of altered TRP protein expression for cancer diagnosis and prognostication is beyond the scope of this Review. However, such altered TRP channel expression may constitute a therapeutic target. For example, high-grade astrocytoma shows increased TRPV1 expression compared with normal brain[281]. High-dose capsaicin administration can kill neurons owing to the Ca^{2+} overload it causes (FIG. 2), and thus TRPV1 agonists may help to eradicate this brain tumour. Unfortunately, it is unclear how to deliver capsaicin to the brain in doses sufficiently high to kill tumour cells without causing unacceptable side effects. Oesophageal and head-and-neck squamous cell carcinomas also overexpress TRPV1 and TRPA1 (REF.[282]). These cancers are more promising targets for TRPV1 and/or TRPA1 agonist therapy inasmuch as they are amenable to topical administration.

TRPV1 gene polymorphism has been linked to eating habits in children[283]. There is anecdotal evidence that dietary capsaicin may help maintain a healthy body weight (‘exercise in a pill’)[284]. Even if true, it is unclear whether this is an on-target effect of capsaicin on TRPV1, or whether is due to capsaicin-induced changes in the gut microbiota[285], as experiments with *Trpv1*$^{-/-}$ mice provided conflicting reports[78].

TRPM5 is a calcium-activated cation channel activated downstream of taste receptors and other chemosensory receptors. Unlike wild-type mice, which overeat chocolate and become obese, *Trpm5*$^{-/-}$ animals maintain their normal weight when on a carbohydrate-rich diet[286]. Interestingly, these animals also consume less alcohol[287], implying value for a TRPM5 antagonist in obesity and/or alcohol use disorder. By contrast, *Trpm8*$^{-/-}$ mice are obese owing to daytime hyperphagia[288], whereas the TRPM8 agonist, icilin, increases energy expenditure, and reduces body weight, in mice[289]. These observations identify TRPM8 as another potential target in diet-induced obesity.

Type 2 diabetes mellitus (T2DM) has reached pandemic proportions. To date, no disease-modifying treatment is available for T2DM patients. Accumulating evidence suggests that the sensory nervous system is involved in the progression of T2DM by maintaining a low-grade inflammation via TRPV1 (REF.[290]). Indeed, oral glucose tolerance and glucose-stimulated insulin secretion were improved by both genetic inactivation (*Trpv1*$^{-/-}$ mice) and pharmacological blockade of TRPV1 by the small molecule antagonist BCTC[291]. The TRPV1 antagonist, XEN-D0501, is currently undergoing phase II clinical trials in T2DM patients with good tolerability and safety[292]. A combined TRPV1 and TRPA1 antagonist may also protect sensory nerves and prevent cognitive decline in T2DM patients (BOX 2).

Conclusions

The huge interest of pharmaceutical companies in TRPV1 antagonists was based on two premises: first, the animal models in which capsaicin desensitization inhibited pain dramatically, and second, the belief that expression of TRPV1 was restricted to nociceptive fibres. Unfortunately, neither has turned out to be true. Those initial animal models are now recognized as poor predictors of clinical efficacy, and TRPV1 expression is much broader than initially thought. Despite these disappointments, the TRP family remains an exciting and potentially rewarding group of therapeutic targets for a broad range of diseases. Clearly, the one-size-fits-all approach is not feasible and tests need to be developed to identify patient subgroups that may benefit from the therapy. Current TRP channel-modulator drug discovery efforts are guided by human genetics to allow identification of specific indications and patient groups that could benefit from pharmacotherapy (BOX 3). Furthermore, appropriate target engagement and safety biomarker studies should help define clinically meaningful doses and therapeutic windows. Central unwanted side effects may be minimized by using peripherally restricted antibodies, antibody–drug conjugates, and engineered proteins, whereas systemic adverse effects can be avoided by targeted, site-specific therapy (BOX 4). We predict that novel treatment modalities, such as engineered proteins, oligonucleotides and gene-based therapies, will be increasingly explored to treat human TRP channel-associated diseases in the future.

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A recent comprehensive analysis of the structural similarities and differences of the transmembrane regions of TRP channels that reveals hot spots for interactions with modulatory chemical agents, including natural agonists, antagonists, tool compounds and drugs.

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