



The emerging role of transient receptor potential channels in chronic lung disease

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Transient receptor potential channels are emerging as novel targets for chronic lung diseases with a high unmet need <http://ow.ly/GHeR30b3hIy>

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ABSTRACT Chronic lung diseases such as asthma, chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis are a major and increasing global health burden with a high unmet need. Drug discovery efforts in this area have been largely disappointing and so new therapeutic targets are needed. Transient receptor potential ion channels are emerging as possible therapeutic targets, given their widespread expression in the lung, their role in the modulation of inflammatory and structural changes and in the production of respiratory symptoms, such as bronchospasm and cough, seen in chronic lung disease.

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Introduction

Chronic lung diseases are a major global healthcare burden and present an unmet need with regard to the availability of effective therapies. There have been relatively few new classes of therapeutics introduced over the past 40 years [1, 2]. Although some new chemical entities have entered the respiratory space, most of the “new” small-molecule treatments have been improvements to existing drugs with the introduction of longer acting inhaled β_2 -adrenoceptor agonists (LABAs) and anticholinergics (LAMAs) and inhaled corticosteroids with an improved therapeutic ratio. Biological therapies have been more successful, at least for allergic airways disease, with the development of IgE and interleukin (IL)-5, IL-4 and IL-13 targeted therapies [3].

Ion channels are attractive drug targets because they play a crucial role in controlling many physiological processes, and because their dysfunction can lead to pathophysiology. Furthermore, in nonrespiratory conditions there is a historical precedent for discovering and commercialising successful drugs that modulate the activity of voltage-gated sodium, calcium or potassium channels, or ligand-gated ion channels [4]. It is not clear why this approach has not extended to respiratory diseases, but recent publications suggest that this is about to change with increasing recognition that modulation of ion channel function may be a successful strategy for modelling the aberrant pathophysiological changes observed in chronic lung diseases.

One of the most studied ion channel families are the transient receptor potential (TRP) family of ion channels. TRP channels were originally discovered in *Drosophila* as phototransduction proteins, and this led to the identification of a large family of proteins [5]. There are 28 mammalian TRP channels, which are subdivided into seven groups based on sequence homology: TRPV (vanilloid), TRPA (ankyrin), TRPM (melastatin), TRPP (polycystin), TRPC (canonical), TRPML (mucolipin) and TRPN (no mechanoreceptor potential C; not expressed in mammals) [6, 7]. TRP channels are cation-selective ion channel proteins with a preference for calcium that are widely expressed in the respiratory tract across a wide range of neuronal and non-neuronal cell types. The focus of this article is the particular TRP channels whose expression profiles and functional effects have been documented within the respiratory tract, namely TRPV1, TRPV4, TRPA1, TRPM8 and TRPM3. We focus on the role of these channels in asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF) and chronic idiopathic cough [8–11]. In the main, TRPs have been studied in the context of the part they play in the activation of airway sensory afferents and the cough reflex, but a role is emerging for TRPs in the broader context of chronic lung disease pathophysiology with a modulatory function in the control of inflammatory and structural cell function. This review summarises what is known about the TRP channel and sensory nerve function and expands on the wider role of TRPs in non-neuronal cells.

Chronic lung disease

Asthma and COPD are both obstructive airways diseases and are characterised by chronic inflammation of the respiratory tract. Both diseases are prevalent and increasing throughout the world. Although asthma can be controlled well with inhaled corticosteroids, adherence to this treatment is poor and inhaler use often inadequate [12]. ~20% of asthma patients are particularly difficult to manage, and 3–5% of patients have severe disease that is not controlled even by maximal-dose inhaled corticosteroid treatment and a LABA [13]. COPD is characterised by a progressive fixed airflow limitation that is often associated with a chronic inflammatory response to inhaled gases or noxious particles, usually cigarette smoke. COPD is considered at diagnosis based on the presence of chronic cough, dyspnoea, sputum production and exposure to known risk factors such as cigarette smoking. No current treatments, including corticosteroids, reduce disease progression or mortality and have relatively little effect (~20% reduction) in preventing exacerbations [14]. Therefore, there is a need to develop more effective therapies.

IPF is a chronic progressive and invariably fatal scarring lung disease with a prognosis <3–5 years, which is worse than many cancers [15]. Although the aetiology and the pathogenesis of IPF are not understood, two antifibrotic drugs, pirfenidone and nintedanib, have recently shown some efficacy in slowing disease progression and are now approved as treatments in the United States and Europe, but the effect of these treatments is relatively small. In addition, patients often deteriorate within months of diagnosis and frequently report debilitating symptoms and a reduced quality of life due to breathlessness and cough. The major activity in the pharmaceutical industry is focused on finding treatments for asthma and COPD based on their prevalence and unmet need. Although IPF is classified as a rare disease, and by definition this means that it is not prevalent, it is in the interest of pharmaceutical companies to obtain orphan designation, because it allows them to apply for clinical development incentives and for fee reductions in regulatory procedures. Consequently, there is a strong drive within many respiratory-focused companies to find effective treatments for IPF [15].

Cough is an important reflex mechanism, whose primary function is to protect the airway from the inhalation of irritant substances. However, excessive cough is a symptom of several chronic lung diseases due to the increased activation of sensory afferent neuronal pathways [16]. Chronic cough can be idiopathic in nature, but is also a major symptom of most chronic lung diseases, including COPD, asthma, IPF and lung cancer, or conditions outside the lung, such as gastro-oesophageal reflux and rhinosinusitis [17]. Chronic cough severe enough to interfere with the normal activities of daily life is thought to affect ~7% of the population [18]. Antitussive medications are one of the most widely sold over-the-counter (OTC) medications, but a systematic review found a lack of evidence to support the use of OTC medicines for the treatment of cough [19]. Despite the high prevalence, treatment of chronic cough is still a significant unmet medical need, and there is an urgent requirement for new, safe and effective therapies.

Role of TRP channels in sensory nerve activation and respiratory reflexes and cough

The cough reflex is regulated by vagal afferent nerves which innervate the airway [8, 20]. The cell bodies of airway sensory nerves are mostly housed in the nodose and jugular ganglia. The nodose and jugular ganglia have different embryonic origins; the nodose is epibranchial placode-derived, and the jugular is neural crest-derived, and as a result they express different proteins (e.g. ion channels or G protein coupled receptors (GPCRs)) and house different populations of nerve fibres. There are several known sensory nerve subtypes present in the lung, which can be identified based on adaptation indices, physiochemical sensitivity, neurochemistry, origin, myelination conduction velocities and sites of termination [8, 21]. Of these sensory nerve subtypes, some are more mechanically sensitive: the rapidly adapting receptors, slowly adapting receptors and the subtype known as the “cough” receptor; and some are more chemosensitive: C fibres and A δ nociceptors. Ion channels present on these vagal nerve termini (and possibly neuronal auxiliary cells) situated in and under the airway epithelium can be activated by a wide variety of stimuli to elicit cough and other reflexes. These include mechanical stimuli such as punctate touch and lung inflation, inflammatory mediators released during disease such as prostaglandin (PG)E₂ and bradykinin, environmental irritants such as cigarette smoke and pollutants, changes in osmolarity of the airways and changes in pH or temperature [8]. The main family of ion channels implicated in the initiation of sensory reflexes are TRP channels. Until recently, TRP channel biology, at least in the respiratory arena, has been mainly focused on their role in sensory nerve function.

TRPV1

TRPV1 is a polymodal ion channel that is activated by diverse stimuli, including the direct activators capsaicin (active constituent of chilli peppers from piquant *Capsicum* species plants), noxious heat >42°C, acidic conditions and protons that interact directly with the channel to cause a lowering of its voltage dependency, leading to opening of the pore domain [22]. It can also be activated indirectly by endogenous “disease-relevant” mediators, including bradykinin and PGE₂ *via* activation of their own GPCRs (B₂ and EP₃, respectively) and secondary signalling pathways not yet clearly defined [8–10, 23]. Although it has been demonstrated that TRPV1 contains domains for binding proteins such as A-kinase-anchoring protein that can influence its activity by facilitating interactions with the signalling cascade kinases protein kinase A and C [24].

The majority of articles so far have focused on the role of capsaicin, and thereby the TRPV1 receptor, in the activation of the cough reflex [25–27]. Interestingly, the tussive activity of capsaicin was noted many years before the identification of TRPV1 [28, 29]. However, subsequently, TRPV1 was discovered by CATERINA *et al.* [22] when it was cloned from rodent dorsal root ganglion neurons and identified as the molecular target for capsaicin. Later, TRPV1 mRNA was also found to be expressed in airway-specific neurons in the vagal ganglia *via* single-cell reverse transcriptase PCR [30, 31]. Dogma states that TRPV1 is expressed on a subset of capsaicin-sensitive slow-conducting unmyelinated C fibres, but it is now known to be more widely expressed on fast-conducting A δ -fibres [32].

A significant amount of information has been gathered regarding the biology of TRPV1 from clinical capsaicin challenge studies. Interestingly, the threshold for provoking cough by capsaicin (often referred to as the C2 or the C5: concentration of capsaicin required to elicit two or five coughs, respectively) has been found to be lowered in various populations of COPD [33–35], asthmatic [33, 35–39], IPF [40] and chronic idiopathic cough [35, 41] patients who have chronic cough compared to healthy controls. These data imply that TRPV1 function is increased in a disease setting. Conceptually, TRPV1 function can be modulated in a respiratory disease setting by 1) elevated levels of the endogenous activating mediator produced by the “diseased” lung; 2) peripheral or central sensitisation of the ion channel so that it responds at lower thresholds; and 3) phenotype change in the nerve leading to increased expression of the ion channel. In fact, increased levels of presumed activators/sensitisers of the channel are found in the lung of asthma and COPD patients and neuronal phenotype change noted in disease-relevant models. For

example, higher concentrations of PGE₂ [42] and bradykinin [43] have been found in the airways of patients with asthma and COPD. Increased PGE₂ levels have been found in idiopathic cough and cough associated with postnasal drip, gastro-oesophageal reflux disease, cough variant asthma and eosinophilic bronchitis [44]. Furthermore, in an animal model of asthma where guinea pigs were sensitised and challenged with ovalbumin or administered neurotrophin there was an increase in the number of TRPV1-positive neurons (particularly of the nodose-originating, Aδ subtype), suggesting that neural pathways innervating the lung can be influenced by their environment to undergo a phenotype change [31]. Similar data have been generated in a rat model of asthma with an increase in TRPV1-expressing neurons in the nodose ganglia [45]. In a recent study, patients with COPD had increased cough responses to capsaicin, but reduced responses to PGE₂ compared with healthy volunteers [35]. Consistent with these findings, capsaicin caused a greater number of coughs in cigarette smoke-exposed guinea pigs than in control animals; similar increased responses to capsaicin were observed in *ex vivo* vagus nerve and neuron cell bodies in the vagal ganglia, although in contrast, PGE₂ responses were decreased by cigarette smoke exposure. These data suggest that cigarette smoke exposure in guinea pigs is capable of inducing a phenotype change in airway sensory nerves comparable with the cough responses observed in cigarette smoke exposed animals and patients with COPD. Interestingly, the same study showed that different patient groups (COPD, healthy smokers, refractory chronic cough and asthma) demonstrated differing patterns of cough response to capsaicin, PGE₂ and citric acid. The different profiles of cough responses support the concept of disease-specific neurophenotypes in chronic lung disease [35]. Interestingly, it is still not clear whether increased sensitivity to various challenge agents acting on particular GPCRs or ion channel targets informs us regarding whether a particular intervention will be useful in a particular disease setting. Currently, the only example is a study which utilised the TRPV1 inhibitor, SB-705498, in a clinical trial in patients with chronic idiopathic cough (a group which demonstrates an increased cough sensitivity to the TRPV1 agonist, capsaicin) utilising objective cough monitoring and capsaicin challenge as a pharmacodynamic marker of target engagement. In this study SB705498 was shown to have a small, statistically significant effect on capsaicin-evoked cough, but no effect on spontaneous cough frequency in refractory chronic cough patients [46]. This study may provide an early indication that increased sensitivity to one mechanism (e.g. TRPV1), demonstrated by challenge studies (e.g. capsaicin), does not necessarily implicate the utility of a certain target in a particular patient group. However, as there was only a small shift in the response to capsaicin, the pharmacodynamic marker in this study, further studies, with a more appropriate interventional tool, are required for confirmation.

TRPA1

TRPA1 (formerly ANKTM1) is the only member of the TRP-ankyrin family of ion channels expressed in mammals. It was named according to the large number of ankyrin repeats on its N-terminal domain [47]. TRPA1 is a voltage-dependent calcium-permeable cation channel which was originally discovered in human cultured fibroblasts [48], but is now known to be widely expressed in sensory nociceptive neurons in the vagal, jugular and nodose ganglia [30, 47]. However, unlike TRPV1, TRPA1 only seems to activate C-fibres and, interestingly, single-cell PCR experiments have identified that although they are often co-expressed in neurons within the jugular and nodose ganglia, they are also found separately [32, 49]. TRPA1 channels are activated by a range of natural products such as allyl isothiocyanate, allicin and cannabinal, found in mustard oil, garlic and cannabis [50–52], and by environmental irritants (e.g. acrolein, present in air pollution and cigarette smoke) [53, 54]. TRPA1 is also the molecular target for reactive and electrophilic by-products of oxidative stress including reactive oxygen species (ROS), nitrate (reactive nitrogen species) and carbonylic (reactive carbonyl species) stress. Interestingly, this also includes electrophiles such as hypochlorite and hydrogen peroxide [55, 56]. Recently, lipopolysaccharide (LPS) was identified as a TRPA1 activator and exerts fast, membrane delimited, excitatory actions *via* TRPA1, which develop independently of TLR4 activation [57]. In addition, recent data have demonstrated an interaction between diesel exhaust particles (DEP) and the activation of airway C-fibre afferents. Polycyclic aromatic hydrocarbons, major constituents of DEP, were implicated in this process *via* activation of the aryl hydrocarbon receptor and subsequent mitochondrial ROS production, which is known to activate TRPA1 on nociceptive C-fibres [58]. In addition to chemical ligands, TRPA1 is believed to be a sensor of physical stimuli, including responding to low noxious cold temperatures <17°C [47], although there is still a certain amount of controversy surrounding the role of TRPA1 as a thermosensor [59, 60].

TRPA1 antagonists have been demonstrated to inhibit sensory nerve activation and cough evoked by TRPA1 agonists and inflammatory mediators such as PGE₂ and bradykinin following activation of their respective GPCRs and cough in response to citric acid (low pH solution) [23, 61–64]. Cinnamaldehyde, a TRPA1 agonist, has been shown to cause cough in clinical studies in normal volunteers [63]. In addition, TRPA1 antagonists have also been shown to significantly inhibit cough in a conscious guinea pig model to the clinical challenge agent, citric acid [63, 64]. However, it is not known whether it is the low pH component of

citric acid or the increased osmolarity that is responsible for activating TRPA1 and evoking cough. Previously, it was commonly believed that citric acid evoked cough by virtue of its low pH properties and activation of TRPV1 and acid-sensing ion channels were believed to be responsible [29, 65, 66].

In allergic asthma, exposure to relevant antigens leads to an early asthmatic response (EAR) followed, in certain subjects, by a corticosteroid-sensitive late asthmatic response (LAR). Although many subjects with asthma consider LAR to be one of the defining symptoms of their disease, and despite its widespread use in the clinical assessment of new therapeutic entities, the mechanism underlying the LAR remains unclear. Recent studies suggest that TRPA1 channels activate vagal bronchopulmonary C-fibres in rodent lung, inducing a LAR in sensitised rodents following allergen challenge [67]. The mechanism of action is not completely elucidated, but it is likely that allergens stimulate the channel indirectly *via* the release of endogenously produced TRPA1 activators (possibly mast cell products such as tryptase released as a consequence of the EAR), which leads to sensory nerve activation *via* the TRPA1 channel and central reflex events, ultimately leading to a parasympathetic, cholinergic reflex bronchoconstrictor response (inhibited by LAMAs such as tiotropium and glycopyrrolate) which may be responsible for the LAR seen in this model [67] (figure 1). The data are supported by clinical studies suggesting that LAMAs can improve symptoms and lung function in patients with asthma [68]. Interestingly, toluene di-isocyanate (TDI), a reactive compound used in the manufacture of polymeric derivatives, can evoke respiratory symptoms, including LAR, in exposed workers [69]. Furthermore, it is believed that TRPA1 activation mediates TDI-induced respiratory irritation, and that in this case the irritant may directly stimulate sensory nerves to initiate a steroid-resistant LAR [70]. Therefore, irritant-induced asthma models may be a good model of nonatopic asthma, and these data suggest that neuronal reflexes may be a feature of this particular asthma phenotype. The same may be true for exercise, as we believe that airway dehydration as a result of increased ventilation resulting in augmented osmolarity of the airway lining fluid may activate TRP channels and thereby airway reflex events. It is well known that hyperosmolar solutions (*e.g.* mannitol) provoke bronchoconstriction and cough in asthmatics *via* activation of sensory nerves [71]. Consistent with a neuronal mechanism, anticholinergics produce significant protection from exercise-induced bronchospasm [72]. With nonallergic triggers we propose that the agent would directly stimulate nerves, *via* TRP channels, to induce bronchospasm/LAR/cough in a steroid-resistant manner, explaining the relative treatment-resistant phenotype associated with nonallergic, non-T-helper cell type 2 asthma (figure 1).

TRPV4

TRPV4 is a calcium- and magnesium-permeable nonselective cation channel with a proline-rich region and six ankyrin repeats within its cytosolic N-terminus [73]. TRPV4 is a temperature-sensitive ion channel responding to temperatures in the range 24–42°C and to a wide range of endogenous and exogenous stimuli (*e.g.* arachidonic acid derivatives, changes in osmolarity, endocannabinoids, α -phorbols, proteases and mechanical stimuli) [74–76]. TRPV4 mRNA expression has been demonstrated in rodent sensory, trigeminal, dorsal root, vestibular, nodose and jugular ganglia [8, 9, 77–81]. However, expression studies were performed on whole ganglia rather than single-cell expression studies on isolated neurons, so expression on non-neuronal cells cannot be ruled out. More recently, single-cell expression studies performed on airway-specific neurons from the jugular or nodose ganglia did not yield convincing data relating to TRPV4 mRNA expression in the vagal ganglia [82], but did demonstrate TRPV4-induced activation of guinea pig airway-specific, primary nodose, but not jugular, ganglion cells. In the same study, TRPV4 ligands and hypo-osmotic solutions caused depolarisation of murine, guinea pig and human vagus nerve and firing of A δ fibres (not C-fibres) which was inhibited by TRPV4 and P2X3 receptor antagonists. Both antagonists blocked TRPV4-induced cough, suggesting that TRPV4 releases ATP which activates the A δ -fibres to induce cough. This study identifies the TRPV4–ATP–P2X3 axis as a driver of airway sensory nerve reflexes such as cough [82]. However, it is still not clear where TRPV4 is expressed in this context, although the identification of P2X3 receptors on airway afferents [83] and a recent clinical trial demonstrating the efficacy of a P2X3 receptor antagonist in treatment-resistant chronic cough patients would support the role of ATP as a driver of the cough reflex [84].

TRPM8

TRPM8 is a member of the melastatin family of nonselective cation channels and functions as a homotetramer [85] and is expressed in a subpopulation of primary afferent neurons within the dorsal root, trigeminal [86], vagal, nodose and jugular ganglia [30]. TRPM8 is activated by the cooling compounds menthol, icilin and eucalyptol and by cool temperatures (15–28°C) [85, 86]. In fact, TRPM8 is thought to be the target responsible for cough and bronchoconstriction caused by inhalation of cold air [86, 87]. In contrast, menthol, which is thought to exert its actions through the TRPM8 channel, has been shown to inhibit citric acid-induced cough in guinea pigs [88, 89] and humans [90] and causes a short-lasting

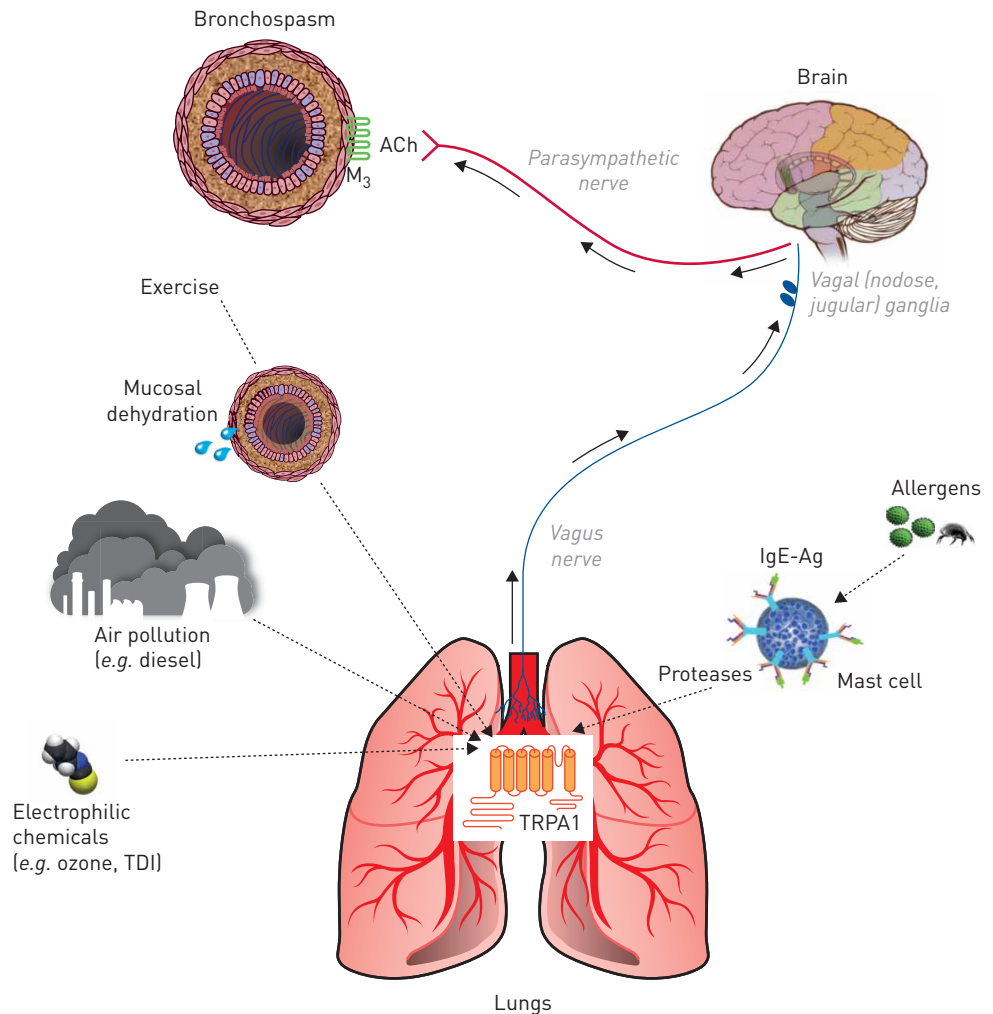


FIGURE 1 Schematic representation of the proposed mechanisms involved in the late asthmatic response (LAR) evoked by allergic and nonallergic stimuli. In allergic asthma, exposure to relevant antigens leads to an early asthmatic response (EAR) followed, in certain subjects, by a corticosteroid-sensitive LAR. Our hypothesis is that allergen challenge leads to sensory nerve activation *via* the transient receptor potential (TRP)A1 channel (blocked by TRPA1 antagonists) and central reflex events (blocked by anaesthesia), ultimately leading to a parasympathetic cholinergic bronchoconstrictor response. This is mediated by acetylcholine (ACh) activating muscarinic M₃ receptors, causing bronchospasm, which is blocked by anticholinergics. The mechanism of action is not completely clear, but it is likely that the allergen stimulates the channel indirectly *via* the release of endogenously produced TRPA1 activators (possibly mast cell products such as tryptase released as a consequence of the EAR). Many nonallergic asthma triggers (e.g. reactive chemicals, ozone, air pollution and exercise) can initiate respiratory symptoms, including a LAR. It is now recognised that TRP channel activation may be involved in the initiation of these reflex events. Many of these agents (e.g. toluene di-isothiocyanate (TDI)) can stimulate TRP channels directly to induce respiratory irritation. Our hypothesis regarding exercise is that the key stimulus is airway dehydration as a result of increased ventilation, resulting in augmented osmolarity of the airway lining fluid. It is well known that hyperosmolar solutions provoke bronchoconstriction, and are known to activate sensory nerves and evoke reflex events such as bronchospasm and cough in asthmatics. Consistent with a neuronal mechanism, anticholinergics provide significant protection from exercise-induced bronchospasm. With nonallergic asthma triggers we propose that the agent could directly stimulate sensory nerves to initiate the LAR in a steroid-resistant manner, explaining the relative treatment-resistant phenotype associated with nonallergic, non-T-helper cell type 2 asthma.

decrease in capsaicin cough in normal subjects [91]. Furthermore, it has been used as an OTC medication for a number of years for its antitussive properties, and is often added to cigarettes to inhibit irritancy [73]. Therefore, there is still a question mark over whether the beneficial effects of menthol in the lung are mediated *via* activation of TRPM8 [92], and there are conflicting data on the role of the TRPM8 channel in the literature. 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 TRPA1 at high concentrations. A number of novel TRPM8 inhibitors have been developed, which should help shed light on the role of this channel.

TABLE 1 Expression profile of transient receptor potential (TRP) channels

	Expression	References
TRPV1		
Human	Epithelial cells	[99–105]
	Lung macrophages	[99]
	T-lymphocytes	[106, 107]
Mouse	Vagal ganglia	[30]
Guinea pig	Vagal ganglia	[31, 49]
Rat	Vagal ganglia	[45]
TRPA1		
Human	Fibroblasts	[48, 108]
	Epithelial cells	[108–110]
	Airway smooth muscle	[110]
Mouse	Vagal ganglia	[30, 47]
Guinea pig	Vagal ganglia	[49]
TRPV4		
Human	Airway smooth muscle	[111–113]
	Fibroblasts	[114]
	Epithelial cells	[99]
	Macrophages	[99]
Guinea pig	Neuronal satellite glia	[82]
TRPM8		
Mouse	Vagal ganglia	[30]
TRPM3		
Guinea pig	Vagal ganglia	[95]

TRPM3

The TRPM3 gene codes for different TRPM3 isoforms with different pore and gating properties [93]. TRPM3 α 2 is a calcium-permeable nonselective cation channel, and is referred to as TRPM3 in this article. TRPM3 has been identified as a noxious heat sensor and TRPM3 knockout mice have an impaired detection of noxious heat [94]. TRPM3 is activated by hypotonic cell swelling [95], the neurosteroid pregnenolone sulphate [96] and the potent synthetic ligand CIM0216 [97]. There is a synergistic effect of heat and pregnenolone sulphate or CIM0216 on the activation of TRPM3 [94, 97]. It is a volume-regulated ion channel shown to be highly expressed in somatosensory neurons [94], and gene expression studies have demonstrated it to be highly expressed in single airway-specific neurons from the jugular and nodose ganglia [98]. Activation of TRPM3 on airway sensory nerves has been shown to cause calcium flux in both the nodose and jugular ganglia, depolarisation of guinea pig, mouse and human vagus nerves and single fibre firing in the anaesthetised guinea pig [98]. Further investigations are required to determine whether activation of TRPM3 results in functional effects in the airways and whether these are of relevance in chronic lung diseases.

Role of TRP channels in non-neuronal cells and in the pathophysiology of chronic lung disease

Emerging data show more widespread expression of TRP channels in the lung on non-neuronal cells, including structural and immune cells [9, 11] (table 1). Furthermore, in some cases certain TRP channel single nucleotide polymorphisms (SNPs) have been associated with “disease” phenotypes and increases in TRP gene expression found in lung samples from patients with chronic lung disease. These data all point to a role for TRP channels in disease pathophysiology in addition to their well-documented role in the control of respiratory symptoms such as bronchospasm and cough [9, 11].

TRPV1

Originally, TRPV1 channel expression was thought to be confined to sensory afferents, but it is now thought to be expressed in a range of tissues and organs. In the lung, TRPV1 expression has been found in human bronchial epithelial cells with an increase in expression in cells from the airways of patients with refractory asthma [99–105, 109], fibroblasts [102], T-cells [106, 107, 115] and human alveolar macrophages [99]. Several studies suggest that TRPV1 channel activation evokes the release of proinflammatory cytokines and ATP from bronchial epithelial cells [99, 103–105, 116]. Furthermore, in the context of COPD, the TRPV1 inhibitor JNJ17203212 reduced the cigarette smoke-induced release of ATP from human bronchial epithelial cells and TRPV1^{-/-} mice demonstrated less cigarette smoke-induced

ATP release and exhibited reduced neutrophilic inflammation in the bronchoalveolar lavage fluid than wild-type mice [99]. Interestingly, in the same study TRPV1 mRNA expression was increased in whole-lung homogenates from emphysema patients compared to smokers and healthy nonsmokers, suggesting increased expression of TRPV1 associated with disease pathophysiology in non-neuronal cell types [99]. Another study investigated the association of SNPs in the candidate genes TRPV1, TRPV4 and TRPA1 with cough symptoms. TRPV1 SNPs were associated with cough in subjects without asthma from two independent studies in eight European countries, suggesting that TRPV1 SNPs may enhance susceptibility to cough in current smokers and in subjects with a history of workplace exposure [117].

Additional data exist in animal models of asthma either utilising small-molecule antagonists or *TRPV1*^{-/-} knockout mice. Some studies have shown no impact or alternatively a protective effect of TRPV1 inhibition in rodent ovalbumin challenge models on standard end-points thought to encapsulate the allergic asthma phenotype including eosinophilia, airway hyperresponsiveness and the LAR [68, 118, 119]. In other studies, inhibition of TRPV1 has been shown to inhibit the IL-13-driven asthma phenotype in a murine model and airway hyperresponsiveness to histamine in a guinea pig ovalbumin model [120, 121]. Consistent with these findings, another study suggests that TRPV1, and not TRPA1, plays a discrete role in modulating aspects of the asthma phenotype in a house dust mite-driven murine model reducing plasma IgE levels, airway cellular inflammation and airway hyperresponsiveness. In this study confirmation was obtained in an alternative model system utilising a second species, the Brown Norway rat, an alternative antigen; ovalbumin and a small-molecule TRPV1 inhibitor, XEN-D0501 [122]. Interestingly, the effect of XEN-D0501 on cellular inflammation, when dosed around the challenge, was similar to that observed in *TRPV1*^{-/-} mice, but the levels of IgE remained unchanged, consistent with an effect in the challenge phase of the model [122]. In addition, an impact was observed on the LAR. Although many of the effects seen in this study did not reach statistical significance there was a consistent suppression across the functional end-points measured, suggesting a discrete role for TRPV1 in driving CD4⁺-dependent allergic asthma models [122].

It is not clear why differences have been observed in studies investigating a role for TRPV1 in allergic asthma, but this could be explained by the differences in species, strain, antigens and interventions used to dissect TRPV1 biology. Given the emergence of potent TRPV1 inhibitors with appropriate pharmacokinetic profiles for *in vivo* studies the picture should become clearer in the future [11]. In the absence of definitive preclinical data, there are data available from human *in vitro* studies where increased TRPV1 gene expression has been demonstrated in asthma [100, 102], and population-based genetic association studies which have documented a TRPV1 genetic variant to be associated with a lower risk of childhood asthma or the presence of wheezing [123].

TRPA1

Although TRPA1 is predominantly expressed in sensory nociceptive neurons, TRPA1 gene expression has been demonstrated in non-neuronal cells, including epithelial, fibroblasts, B-cells, airway smooth muscle, CD4⁺ and CD8⁺ T-cells [108, 109, 110, 124]. A role for TRPA1 has been demonstrated in murine models of allergic asthma utilising small molecule antagonists and *TRPA1*^{-/-} mice with an inhibitory effect on inflammatory mediator and mucus production, leukocyte infiltration and airway hyperresponsiveness (AHR) to acetylcholine [118]. In addition, *TRPA1*^{-/-} mice displayed deficiencies in allergen-induced neuropeptide release in the airways, which the authors suggested provided a potential explanation for the impaired inflammatory response, although the rationale for this is not completely clear and this finding was not reproduced in other studies [122]. Interestingly, a role for TRPA1 has also been proposed in nonallergic inflammation in a study set up in a murine model which aimed to recapitulate incidents of AHR experienced by swimmers due to increased exposure to hypochlorite. The authors proposed that the AHR following exposure to hypochlorite and ovalbumin depends on a neuroimmune interaction that involves TRPA1-dependent stimulation of sensory neurons and mast cell activation, although the rationale is not obvious given AHR to direct contractile agents is not thought to have a neuronal component as they are thought to activate airway smooth muscle directly [125]. Another article by the same authors demonstrated that TRPA1, TRPV1 and mast cells play an indispensable role in the development of TDI-elicited AHR [126]. In the context of COPD-relevant disease biology, cigarette smoke extract has been shown to evoke IL-8 release from human epithelial cells, smooth muscle cells and fibroblasts and cigarette smoke and acrolein have been shown to release keratinocyte-derived chemokine (murine orthologue) in a murine model. These effects were reduced by a TRPA1 antagonist, suggesting a role for TRPA1 in a non-neuronally driven inflammatory response [110]. However, although these observations exist, most of the current literature has pointed to a predominant role for TRPA1 in the activation of sensory afferents and reflex events.

In population-based studies, data have been published demonstrating associations between TRPA1 SNPs and current doctor-diagnosed asthma and total IgE concentration at 7.5 years in the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort. In ALSPAC, there was strong evidence for association between six SNPs and asthma: in a meta-analysis across ALSPAC and two further birth cohorts, the pooled effect estimates confirmed that all six SNPs were significantly associated with asthma. This study suggests that TRPA1 may play a role in the development of childhood asthma [127].

TRPV4

TRPV4 is expressed across a wide range of non-neuronal human cell types in the airways, including structural cells such as airway smooth muscle, epithelial cells, fibroblasts and pulmonary vessels and inflammatory cells including macrophages (but not highly differentiated surrogates: the circulating monocytic precursor cell, monocyte derived macrophages, *in vitro* differentiated promonocytic THP-1 and U937 cells or promyelocytic HL-60 leukaemia cells), neutrophils and T-cells [99, 107, 111, 112, 114, 128–132]. However, in the respiratory system, the function of TRPV4 is probably best understood in relation to its role in acute lung injury, which is not covered in this review [133]. A wide range of proinflammatory, disease-relevant stimuli are known to activate TRPV4, so it is not surprising that activation may give rise to functional effects that could play a role in the pathophysiology of chronic lung diseases. For example, it has been demonstrated that TRPV4 agonists can evoke bronchospasm in an *in vivo* anaesthetised guinea pig model, although in this case sensory nerve reflexes are not involved since both vagus nerves were cut [134]. Consistent with this are observations that a TRPV4 agonist can evoke a mast-cell dependent contractile response of human bronchi and guinea pig tracheal airway smooth muscle *in vitro*. This effect is thought to be mediated by TRPV4-mediated ATP release from airway smooth muscle, which activates P2X4 receptors on mast cells to release cysteinyl leukotrienes (LT) which activate the Cys LT₁ receptor to evoke contraction [112, 134]. Interestingly, many of the functional effects of TRPV4 activation are due to the release of ATP and activation of purinoceptors [8, 82, 99, 134, 135] and TRPV4-induced ATP release has been demonstrated in human macrophages, epithelial and smooth muscle cells [99, 113, 135]. In addition, TRPV4-induced ATP release has been shown to be released *via* a pannexin 1-dependent mechanism [82, 99, 135].

COPD is an inflammatory disease usually associated with cigarette smoking, in which increase in extracellular (e)ATP has been noted and suggested to play a key role in driving cigarette smoke-induced airway inflammation, but the mechanism involved in ATP release has eluded researchers [136, 137]. Cigarette smoke exposure was found to cause a dose-related increase in ATP from primary human bronchial epithelial cells that was attenuated by blockers of TRPV1, TRPV4 and pannexin-1 channels (but not TRPA1) [99]. The same study documented an increase in TRPV4 mRNA expression in whole-lung tissue from COPD patients compared to healthy smokers and nonsmokers. Parallel data were obtained using a murine acute cigarette smoke-driven *in vivo* model system where the neutrophilic inflammatory response was inhibited in *TRPV4*^{-/-} and *pannexin 1*^{-/-} (but not *TRPA1*^{-/-}) mice [99]. These data are consistent with data suggesting that the cigarette smoke-induced inflammation is driven by the release of eATP resulting in P2X7 and subsequent inflammasome activation. Interestingly, the innate immune response was not altered in *TRPV4*^{-/-} mice challenged with an aerosol of LPS [138–141].

Preclinical studies have indicated a possible role for TRPV4 in the pathophysiology of asthma. In a Brown Norway rat ovalbumin model of allergic asthma, antigen challenge triggered a LAR response that was inhibited by two structurally distinct LAMAs, (tiotropium and glycopyrrolate). In naïve rats, an inhaled TRPV4 agonist caused a “LAR-like” response which was attenuated by a TRPV4 inhibitor, a P2X3 inhibitor and glycopyrrolate, suggesting that stimulation of the TRPV4-ATP axis could replicate the LAR. To confirm a role for TRPV4-ATP axis, blockade of TRPV4 or P2X3 attenuated the LAR in the rat ovalbumin asthma model. Furthermore, targeting TRPV4 using either genetically modified mice (house dust mite model) or a pharmacological inhibitor (dosed after sensitisation but before challenge in a rat ovalbumin model) abolished IgE levels in the mouse model, but had no impact in the rat. In addition, airway inflammation was significantly decreased in both models utilising *TRPV4*^{-/-} mice or TRPV4 inhibitors in the rat and this was associated with suppression of antigen-triggered changes in AHR. These data suggest that airway sensory nerves can be triggered *via* a TRPV4-ATP-P2X3 axis to cause a parasympathetic reflex to drive the LAR, and that the TRPV4 axis is responsible for the allergic inflammatory response and AHR, although a role for ATP and any involvement of a P2X receptor remains to be established [142, 143].

TRPV4 channels are expressed and TRPV4 activity is upregulated in lung fibroblasts derived from patients with IPF. In addition, TRPV4-deficient mice are protected from fibrosis [114]. TRPV4 activity modulated transforming growth factor-β1-dependent actions in a SMAD-independent manner with enhanced actomyosin remodelling and increased nuclear translocation of the α-smooth muscle actin transcription

coactivator. These data indicate that TRPV4 activity mediates pulmonary fibrosis and suggest that inhibition of TRPV4 has potential as a therapeutic approach in IPF [114]. As in both asthma and COPD, increased amounts of ATP have been detected in the airways of IPF patients [136–138], but currently there is no evidence available regarding the potential role of the TRPV4–pannexin–ATP axis in IPF disease pathogenesis.

Genetic variants of TRPV4 have been associated with asthma and COPD. A study has described the association of a functional SNP, TRPV4-P19S, with childhood asthma. However, functional analysis of TRPV4-P19S, despite its loss-of-channel function, showed no significant association with asthma or the presence of wheezing [123]. Conversely, a genome-wide association study highlighted seven TRPV4 SNPs which conferred increased susceptibility to COPD pathologies [141]. Therefore, modulating TRPV4 protein structure and activity may impact on COPD pathophysiology. However, further functional studies are needed to clarify the molecular mechanism of TRPV4 variants in the pathophysiology of COPD.

Summary

It is becoming clear that TRP channels have a major role to play in pathophysiology and symptoms across a wide range of chronic lung diseases. Some TRP channels with widespread expression profiles across a number of non-neuronal cell types (*e.g.* TRPV4) may be more broad-spectrum targets capable of impacting the inflammatory phenotypes observed in chronic lung diseases in addition to addressing symptoms such as cough. However, even if the development of an antitussive therapy is not the primary aim, it should be noted that the use of objective cough monitoring as a functional biomarker may be a useful pharmacodynamic marker in early stage proof-of-concept clinical studies in a clinical development programme.

Alternatively, some targets appear to have a more discrete expression profile (*e.g.* TRPA1) which is mostly associated with sensory nerves and as such may be more appropriate to target symptoms such as cough. That said, the role of airway innervation in clinical disease has been largely overlooked and there is now strong evidence to suggest that nerves play a key role in driving disease pathophysiology in addition to symptoms such as cough. An example of this could be nonallergic asthma, given that many of the irritants that trigger asthma episodes among this patient group are now known to activate TRPA1. However, the divergent profile of tussive responses described in clinical challenge studies among patients with different chronic lung diseases and even in patients within one disease group (*e.g.* asthma) supports the hypothesis of disease-specific neurophenotypes [35, 38]. As such it will become necessary to identify which patient subgroups may be more susceptible to therapeutics targeting neuronal hyperresponsiveness. Therefore, it will become increasingly important to perform mechanistic studies preclinically and clinically in order to discover which disease groups are more likely to benefit from certain treatments.

TABLE 2 Preclinical and clinical studies that have utilised transient receptor potential (TRP) receptor antagonists in animal models or in clinical trials for lung indications

	Antagonist	Stage	Study [Reference]/[ClinicalTrials.gov identifier]
TRPV1	JNJ17203212	Preclinical	[23]
	SB705498	Phase 2 (terminated)	Patients with chronic idiopathic cough [46]
	Xention-D0501	Phase 2 (ongoing)	Patients with cough in COPD (NCT02233686) Patients with chronic idiopathic cough (NCT02233699)
TRPA1	HC030031	Preclinical	[23, 61, 62]
	GRC-17536	Preclinical Phase 2	[64] Chronic refractory cough [clinicaltrialsregister.eu identifier 2013-002728-17]
TRPV4	GSK2193874	Preclinical	[82]
	GSK2798745	Phase I safety	A first time in human study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of GSK2798745 in healthy subjects and stable heart failure patients (NCT02119260)
		Phase 2	A study to evaluate the effect of the transient receptor potential vanilloid 4 (TRPV4) channel blocker, GSK2798745, on pulmonary gas transfer and respiration in patients with congestive heart failure (NCT02497937)

COPD: chronic obstructive pulmonary disease.

Various TRP channel antagonists have been developed, predominantly for pain indications (e.g. dental pain, perioperative pain and painful diabetic neuropathy), but have failed in clinical trials. With appropriate compounds available for clinical trials (e.g. AF-219 and TRP antagonists listed in table 2), proof-of-concept clinical studies could be undertaken by adopting a repositioning approach and utilising compounds from pain and other therapeutic areas. This strategy was successful with the P2X3 inhibitor, AF219, which was found to demonstrate unprecedented efficacy in chronic cough in treatment-resistant patients [86]. The success of an oral small-molecule compound in the respiratory area is a breath of fresh air to this field and has given us hope with a new era of ion channel discovery in the respiratory area.

References

- Gibson GJ, Loddenkemper R, Sibille Y, *et al.*, eds. The European Lung White Book: Respiratory Health and Disease in Europe. Sheffield, European Respiratory Society, 2013.
- Barnes PJ, Bonini S, Seeger W, *et al.* Barriers to new drug development in respiratory disease. *Eur Respir J* 2015; 45: 1197–1207.
- Mitchell PD, El-Gammal AI, O'Byrne PM. Emerging monoclonal antibodies as targeted innovative therapeutic approaches to asthma. *Clin Pharmacol Ther* 2016; 99: 38–48.
- Kaczorowski GJ, McManus OB, Priest BT, *et al.* Ion channels as drug targets: the next GPCRs. *J Gen Physiol* 2008; 131: 399–405.
- Montell C, Rubin GM. Molecular characterization of the *Drosophila trp* locus: a putative integral membrane protein required for phototransduction. *Neuron* 1989; 2: 1313–1323.
- Clapham DE. TRP channels as cellular sensors. *Nature* 2003; 426: 517–524.
- De Logu F, Patacchini R, Fontana G, *et al.* TRP functions in the broncho-pulmonary system. *Semin Immunopathol* 2016; 38: 321–329.
- Bonvini SJ, Birrell MA, Smith JA, *et al.* Targeting TRP channels for chronic cough: from bench to bedside. *Naunyn Schmiedeberg's Arch Pharmacol* 2015; 388: 401–420.
- Grace MS, Baxter M, Dubuis E, *et al.* Transient receptor potential (TRP) channels in the airway: role in airway disease. *Br J Pharmacol* 2014; 171: 2593–2607.
- Grace MS, Dubuis E, Birrell MA, *et al.* Pre-clinical studies in cough research: role of transient receptor potential (TRP) channels. *Pulm Pharmacol Ther* 2013; 26: 498–507.
- Wortley MA, Birrell MA, Belvisi MG. Drugs affecting TRP channels. *Handb Exp Pharmacol* 2017; 237: 213–241.
- Partridge MR, van der Molen T, Myrseth SE, *et al.* Attitudes and actions of asthma patients on regular maintenance therapy: the INSPIRE study. *BMC Pulm Med* 2006; 6: 13.
- Chung KF, Wenzel S, European Respiratory Society/American Thoracic Society Severe Asthma International Guidelines Task Force. From the authors: International European Respiratory Society/American Thoracic Society guidelines on severe asthma. *Eur Respir J* 2014; 44: 1378–1379.
- Barnes PJ. New anti-inflammatory targets for chronic obstructive pulmonary disease. *Nat Rev Drug Discov* 2013; 12: 543–559.
- Daccord C, Maher TM. Recent advances in understanding idiopathic pulmonary fibrosis. *F1000Res* 2016; 5: 1046.
- Young EC, Smith JA. Pharmacologic therapy for cough. *Curr Opin Pharmacol* 2011; 11: 224–230.
- Haque RA, Usmani OS, Barnes PJ. Chronic idiopathic cough: a discrete clinical entity? *Chest* 2005; 127: 1710–1713.
- Ford AC, Forman D, Moayyedi P, *et al.* Cough in the community: a cross sectional survey and the relationship to gastrointestinal symptoms. *Thorax* 2006; 61: 975–979.
- Schroeder K, Fahey T. Systematic review of randomised controlled trials of over the counter cough medicines for acute cough in adults. *BMJ* 2002; 324: 329–331.
- Canning BJ, Mori N, Mazzone SB. Vagal afferent nerves regulating the cough reflex. *Respir Physiol Neurobiol* 2006; 152: 223–242.
- Adcock JJ, Douglas GJ, Garabette M, *et al.* RSD931, a novel anti-tussive agent acting on airway sensory nerves. *Br J Pharmacol* 2003; 138: 407–416.
- Caterina MJ, Schumacher MA, Tominaga M, *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997; 389: 816–824.
- Grace M, Birrell MA, Dubuis E, *et al.* Transient receptor potential channels mediate the tussive response to prostaglandin E₂ and bradykinin. *Thorax* 2012; 67: 891–900.
- Veldhuis NA, Poole DP, Grace M, *et al.* The G protein-coupled receptor-transient receptor potential channel axis: molecular insights for targeting disorders of sensation and inflammation. *Pharmacol Rev* 2015; 67: 36–73.
- Collier JG, Fuller RW. Capsaicin inhalation in man and the effects of sodium cromoglycate. *Br J Pharmacol* 1984; 81: 113–117.
- Forsberg K, Karlsson JA. Cough induced by stimulation of capsaicin-sensitive sensory neurons in conscious guinea-pigs. *Acta Physiol Scand* 1986; 128: 319–320.
- Laude EA, Higgins KS, Morice AH. A comparative study of the effects of citric acid, capsaicin and resiniferatoxin on the cough challenge in guinea-pig and man. *Pulm Pharmacol* 1993; 6: 171–175.
- Belvisi MG, Miura M, Stretton D, *et al.* Capsazepine as a selective antagonist of capsaicin-induced activation of C-fibres in guinea-pig bronchi. *Eur J Pharmacol* 1992; 215: 341–344.
- Lalloo UG, Fox AJ, Belvisi MG, *et al.* Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs. *J Appl Physiol* 1995; 79: 1082–1087.
- Nassenstein C, Kwong K, Taylor-Clark T, *et al.* Expression and function of the ion channel TRPA1 in vagal afferent nerves innervating mouse lungs. *J Physiol* 2008; 586: 1595–1604.
- Lieu TM, Myers AC, Meeker S, *et al.* TRPV1 induction in airway vagal low-threshold mechanosensory neurons by allergen challenge and neurotrophic factors. *Am J Physiol Lung Cell Mol Physiol* 2012; 302: L941–L948.
- Adcock JJ, Birrell MA, Maher SA, *et al.* Making sense of sensory nerves: an *in vivo* characterisation of Aδ- and C-fibres innervating guinea pig airways. *Am J Respir Crit Care Med* 2014; 189: A3969.

- 33 Doherty MJ, Mister R, Pearson MG, *et al.* Capsaicin responsiveness and cough in asthma and chronic obstructive pulmonary disease. *Thorax* 2000; 55: 643–649.
- 34 Blanc FX, Macedo P, Hew M, *et al.* Capsaicin cough sensitivity in smokers with and without airflow obstruction. *Respir Med* 2009; 103: 786–790.
- 35 Belvisi MG, Birrell MA, Khalid S, *et al.* Neurophenotypes in airway diseases. Insights from translational cough studies. *Am J Respir Crit Care Med* 2016; 193: 1364–1372.
- 36 Fujimura M, Kamio Y, Hashimoto T, *et al.* Airway cough sensitivity to inhaled capsaicin and bronchial responsiveness to methacholine in asthmatic and bronchitic subjects. *Respirology* 1998; 3: 267–272.
- 37 Weinfeld D, Ternesten-Hasséus E, Löwhagen O, *et al.* Capsaicin cough sensitivity in allergic asthmatic patients increases during the birch pollen season. *Ann Allergy Asthma Immunol* 2002; 89: 419–424.
- 38 Satia I, Tsamandouras N, Holt K, *et al.* Capsaicin-evoked cough responses in asthmatic patients: evidence for airway neuronal dysfunction. *J Allergy Clin Immunol* 2017; 139: 771–779.
- 39 Ekstrand Y, Ternesten-Hasséus E, Arvidsson M, *et al.* Sensitivity to environmental irritants and capsaicin cough reaction in patients with a positive methacholine provocation test before and after treatment with inhaled corticosteroids. *J Asthma* 2011; 48: 482–489.
- 40 Hope-Gill BD, Hildrup S, Davies C, *et al.* A study of the cough reflex in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2003; 168: 995–1002.
- 41 Choudry NB, Fuller RW. Sensitivity of the cough reflex in patients with chronic cough. *Eur Respir J* 1992; 5: 296–300.
- 42 Profita M, Sala A, Bonanno A, *et al.* Increased prostaglandin E₂ concentrations and cyclooxygenase-2 expression in asthmatic subjects with sputum eosinophilia. *J Allergy Clin Immunol* 2003; 112: 709–716.
- 43 Baumgarten CR, Lehmkuhl B, Henning R, *et al.* Bradykinin and other inflammatory mediators in BAL-fluid from patients with active pulmonary inflammation. *Agents Actions Suppl* 1992; 38: 475–481.
- 44 Birring SS, Parker D, Brightling CE, *et al.* Induced sputum inflammatory mediator concentrations in chronic cough. *Am J Respir Crit Care Med* 2004; 169: 15–19.
- 45 Zhang G, Lin RL, Wiggers M, *et al.* Altered expression of TRPV1 and sensitivity to capsaicin in pulmonary myelinated afferents following chronic airway inflammation in the rat. *J Physiol* 2008; 586: 5771–5786.
- 46 Khalid S, Murdoch R, Newlands A, *et al.* Transient receptor potential vanilloid 1 (TRPV1) antagonism in patients with refractory chronic cough: a double-blind randomized controlled trial. *J Allergy Clin Immunol* 2014; 134: 56–62.
- 47 Story GM, Peier AM, Reeve AJ, *et al.* ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* 2003; 112: 819–829.
- 48 Jaquemar D, Schenker T, Trueb B. An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. *J Biol Chem* 1999; 274: 7325–7333.
- 49 Wortley MA, Dubuis E, Bonvini SJ, *et al.* Making sense of sensory nerves: an *in vitro* characterisation of gene expression profiles of airway-innervating guinea-pig airway neurons using single-cell analysis. *Am J Respir Crit Care Med* 2016; 193: A6002.
- 50 Jordt SE, Bautista DM, Chuang HH, *et al.* Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 2004; 427: 260–265.
- 51 Macpherson LJ, Geierstanger BH, Viswanath V, *et al.* The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. *Curr Biol* 2005; 15: 929–934.
- 52 Macpherson LJ, Dubin AE, Evans MJ, *et al.* Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* 2007; 445: 541–545.
- 53 André E, Campi B, Materazzi S, *et al.* Cigarette smoke-induced neurogenic inflammation is mediated by α , β -unsaturated aldehydes and the TRPA1 receptor in rodents. *J Clin Invest* 2008; 118: 2574–2582.
- 54 Bautista DM, Jordt SE, Nikai T, *et al.* TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* 2006; 124: 1269–1282.
- 55 Bessac BF, Sivula M, von Hehn CA, *et al.* TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J Clin Invest* 2008; 118: 1899–1910.
- 56 Taylor-Clark TE. Role of reactive oxygen species and TRP channels in the cough reflex. *Cell Calcium* 2016; 60: 155–162.
- 57 Meseguer V, Alpizar YA, Luis E, *et al.* TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. *Nat Commun* 2014; 5: 3125.
- 58 Robinson RK, Birrell MA, Adcock JJ, *et al.* Mechanistic link between diesel exhaust particles and respiratory reflexes. *J Allergy Clin Immunol* 2017 [In press DOI: <http://doi.org/10.1016/j.jaci.2017.04.038>].
- 59 Chen J, Joshi SK, DiDomenico S, *et al.* Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. *Pain* 2011; 152: 1165–1172.
- 60 Chen J, Kang D, Xu J, *et al.* Species differences and molecular determinant of TRPA1 cold sensitivity. *Nat Commun* 2013; 4: 2501.
- 61 Birrell MA, Belvisi MG, Grace M, *et al.* TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 2009; 180: 1042–1047.
- 62 André E, Gatti R, Trevisani M, *et al.* Transient receptor potential ankyrin receptor 1 is a novel target for pro-tussive agents. *Br J Pharmacol* 2009; 158: 1621–1628.
- 63 Daller JR, Wong J, Brooks BD, *et al.* An inexpensive system for evaluating the tussive and anti-tussive properties of chemicals in conscious, unrestrained guinea pigs. *J Pharmacol Toxicol Methods* 2012; 66: 232–237.
- 64 Mukhopadhyay I, Kulkarni A, Aranake S, *et al.* Transient receptor potential ankyrin 1 receptor activation *in vitro* and *in vivo* by pro-tussive agents: GRC 17536 as a promising anti-tussive therapeutic. *PLoS One* 2014; 9: e97005.
- 65 Canning BJ, Farmer DG, Mori N. Mechanistic studies of acid-evoked coughing in anesthetized guinea pigs. *Am J Physiol Regul Integr Comp Physiol* 2006; 291: R454–R463.
- 66 Trevisani M, Milan A, Gatti R, *et al.* Antitussive activity of iodo-resiniferatoxin in guinea pigs. *Thorax* 2004; 59: 769–772.
- 67 Raemdonck K, de Alba J, Birrell MA, *et al.* A role for sensory nerves in the late asthmatic response. *Thorax* 2012; 67: 19–25.

- 68 Peters SP, Kunselman SJ, Icitovic N, *et al.* Tiotropium bromide step-up therapy for adults with uncontrolled asthma. *N Engl J Med* 2010; 363: 1715–1726.
- 69 Fabbri LM, Chiesura-Corona P, Dal Vecchio L, *et al.* Prednisone inhibits late asthmatic reactions and the associated increase in airway responsiveness induced by toluene-diisocyanate in sensitized subjects. *Am Rev Respir Dis* 1985; 132: 1010–1014.
- 70 Taylor-Clark TE, Kiros F, Carr MJ, *et al.* Transient receptor potential ankyrin 1 mediates toluene diisocyanate-evoked respiratory irritation. *Am J Respir Cell Mol Biol* 2009; 40: 756–762.
- 71 Spector S. Use of mannitol inhalation challenge in assessment of cough. *Lung* 2010; 188: Suppl. 1, S99–S103.
- 72 Spooner CH, Spooner GR, Rowe BH. Mast-cell stabilising agents to prevent exercise-induced bronchoconstriction. *Cochrane Database Syst Rev* 2003; 4: CD002307.
- 73 Nilius B, Szallasi A. Transient receptor potential channels as drug targets: from the science of basic research to the art of medicine. *Pharmacol Rev* 2014; 66: 676–814.
- 74 Nilius B, Vriens J, Prenen J, *et al.* TRPV4 calcium entry channel: a paradigm for gating diversity. *Am J Physiol Cell Physiol* 2004; 286: C195–C205.
- 75 Liedtke W. Transient receptor potential vanilloid channels functioning in transduction of osmotic stimuli. *J Endocrinol* 2006; 191: 515–523.
- 76 Alessandri-Haber N, Yeh JJ, Boyd AE, *et al.* Hypotonicity induces TRPV4-mediated nociception in rat. *Neuron* 2003; 39: 497–511.
- 77 O’Neil RG, Heller S. The mechanosensitive nature of TRPV channels. *Pflügers Arch* 2005; 451: 193–203.
- 78 Kamakura T, Kondo M, Koyama Y, *et al.* Functional expression of an osmosensitive cation channel, transient receptor potential vanilloid 4, in rat vestibular ganglia. *Audiol Neurotol* 2016; 21: 268–274.
- 79 Grant AD, Cottrell GS, Amadesi S, *et al.* Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. *J Physiol* 2007; 578: 715–733.
- 80 Alessandri-Haber N, Dina OA, Joseph EK, *et al.* A transient receptor potential vanilloid 4-dependent mechanism of hyperalgesia is engaged by concerted action of inflammatory mediators. *J Neurosci* 2006; 26: 3864–3874.
- 81 Ni D, Gu Q, Hu HZ, *et al.* Thermal sensitivity of isolated vagal pulmonary sensory neurons: role of transient receptor potential vanilloid receptors. *Am J Physiol Regul Integr Comp Physiol* 2006; 291: R541–R550.
- 82 Bonvini SJ, Birrell MA, Grace MS, *et al.* Transient receptor potential cation channel, subfamily V, member 4 and airway sensory afferent activation: role of adenosine triphosphate. *J Allergy Clin Immunol* 2016; 138: 249–261.
- 83 Kwong K, Kollarik M, Nassenstein C, *et al.* P2X2 receptors differentiate placodal vs. neural crest C-fiber phenotypes innervating guinea pig lungs and esophagus. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L858–L865.
- 84 Abdulqawi R, Dockry R, Holt K, *et al.* P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet* 2015; 385: 1198–1205.
- 85 McKemy DD, Neuhauser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 2002; 416: 52–58.
- 86 Peier AM, Moqrich A, Hergarden AC, *et al.* A TRP channel that senses cold stimuli and menthol. *Cell* 2002; 108: 705–715.
- 87 Xing H, Ling JX, Chen M, *et al.* TRPM8 mechanism of autonomic nerve response to cold in respiratory airway. *Mol Pain* 2008; 4: 22.
- 88 Laude EA, Morice AH, Grattan TJ. The antitussive effects of menthol, camphor and cineole in conscious guinea-pigs. *Pulm Pharmacol* 1994; 7: 179–184.
- 89 Plevkova J, Kollarik M, Poliacsek I, *et al.* The role of trigeminal nasal TRPM8-expressing afferent neurons in the antitussive effects of menthol. *J Appl Physiol* 2013; 115: 268–274.
- 90 Morice AH, Marshall AE, Higgins KS, *et al.* Effect of inhaled menthol on citric acid induced cough in normal subjects. *Thorax* 1994; 49: 1024–1026.
- 91 Wise PM, Breslin PAS, Dalton P. Sweet taste and menthol increase cough reflex thresholds. *Pulm Pharmacol Ther* 2012; 25: 236–241.
- 92 Maher SA, Birrell MA, Bonvini S, *et al.* Beneficial effects of menthol are mediated via a TRPM8-independent mechanism. *Eur Respir J* 2015; 46: Suppl. 59, OA3254.
- 93 Oberwinkler J, Lis A, Giehl KM, *et al.* Alternative splicing switches the divalent cation selectivity of TRPM3 channels. *J Biol Chem* 2005; 280: 22540–22548.
- 94 Vriens J, Owsianik G, Hofmann T, *et al.* TRPM3 is a nociceptor channel involved in the detection of noxious heat. *Neuron* 2011; 70: 482–494.
- 95 Lee N, Chen J, Sun L, *et al.* Expression and characterization of human transient receptor potential melastatin 3 (hTRPM3). *J Biol Chem* 2003; 278: 20890–20897.
- 96 Wagner TFJ, Loch S, Lambert S, *et al.* Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic β cells. *Nat Cell Biol* 2008; 10: 1421–1430.
- 97 Held K, Kichko T, De Clercq K, *et al.* Activation of TRPM3 by a potent synthetic ligand reveals a role in peptide release. *Proc Natl Acad Sci USA* 2015; 112: E1363–E1372.
- 98 Bonvini S, Arshad M, Wortley MA, *et al.* TRPM3: a regulator of airway sensory nerves and respiratory reflexes. *Eur Respir J* 2016; 48: PA5070.
- 99 Baxter M, Eltom S, Dekkak B, *et al.* Role of transient receptor potential and pannexin channels in cigarette smoke-triggered ATP release in the lung. *Thorax* 2014; 69: 1080–1089.
- 100 McGarvey LP, Butler CA, Stokesberry S, *et al.* Increased expression of bronchial epithelial transient receptor potential vanilloid 1 channels in patients with severe asthma. *J Allergy Clin Immunol* 2014; 133: 704–712.
- 101 Yu H, Li Q, Zhou X, *et al.* Transient receptor potential vanilloid 1 receptors mediate acid-induced mucin secretion via Ca^{2+} influx in human airway epithelial cells. *J Biochem Mol Toxicol* 2012; 26: 179–186.
- 102 Sadofsky LR, Ramachandran R, Crow C, *et al.* Inflammatory stimuli up-regulate transient receptor potential vanilloid-1 expression in human bronchial fibroblasts. *Exp Lung Res* 2012; 38: 75–81.
- 103 Jia X, Zhang H, Cao X, *et al.* Activation of TRPV1 mediates thymic stromal lymphopoietin release via the Ca^{2+} /NFAT pathway in airway epithelial cells. *FEBS Lett* 2014; 588: 3047–3054.
- 104 Reilly CA, Taylor JL, Lanza DL, *et al.* Capsaicinoids cause inflammation and epithelial cell death through activation of vanilloid receptors. *Toxicol Sci* 2003; 73: 170–181.

- 105 Reilly CA, Johansen ME, Lanza DL, *et al.* Calcium-dependent and independent mechanisms of capsaicin receptor (TRPV1)-mediated cytokine production and cell death in human bronchial epithelial cells. *J Biochem Mol Toxicol* 2005; 19: 266–275.
- 106 Bertin S, Aoki-Nonaka Y, de Jong PR, *et al.* The ion channel TRPV1 regulates the activation and proinflammatory properties of CD4⁺ T cells. *Nat Immunol* 2014; 15: 1055–1063.
- 107 Majhi RK, Sahoo SS, Yadav M, *et al.* Functional expression of TRPV channels in T cells and their implications in immune regulation. *FEBS J* 2015; 282: 2661–2681.
- 108 Mukhopadhyay I, Gomes P, Aranake S, *et al.* Expression of functional TRPA1 receptor on human lung fibroblast and epithelial cells. *J Recept Signal Transduct Res* 2011; 31: 350–358.
- 109 Büch TR, Schäfer EA, Demmel MT, *et al.* Functional expression of the transient receptor potential channel TRPA1, a sensor for toxic lung inhalants, in pulmonary epithelial cells. *Chem Biol Interact* 2013; 206: 462–471.
- 110 Nassini R, Pedretti P, Moretto N, *et al.* Transient receptor potential ankyrin 1 channel localized to non-neuronal airway cells promotes non-neurogenic inflammation. *PLoS One* 2012; 7: e42454.
- 111 Jia Y, Wang X, Varty L, *et al.* Functional TRPV4 channels are expressed in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2004; 287: L272–L278.
- 112 McAlexander MA, Luttmann MA, Hunsberger GE, *et al.* Transient receptor potential vanilloid 4 activation constricts the human bronchus *via* the release of cysteinyl leukotrienes. *J Pharmacol Exp Ther* 2014; 349: 118–125.
- 113 Takahara N, Ito S, Furuya K, *et al.* Real-time imaging of ATP release induced by mechanical stretch in human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2014; 51: 772–782.
- 114 Rahaman SO, Grove LM, Paruchuri S, *et al.* TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice. *J Clin Invest* 2014; 124: 5225–5238.
- 115 Bertin S, de Jong PR, Jefferies WA, *et al.* Novel immune function for the TRPV1 channel in T lymphocytes. *Channels* 2014; 8: 479–480.
- 116 Seki N, Shirasaki H, Kikuchi M, *et al.* Capsaicin induces the production of IL-6 in human upper respiratory epithelial cells. *Life Sci* 2007; 80: 1592–1597.
- 117 Smit LA, Kogevinas M, Antó JM, *et al.* Transient receptor potential genes, smoking, occupational exposures and cough in adults. *Respir Res* 2012; 13: 26.
- 118 Caceres AI, Brackmann M, Elia MD, *et al.* A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci USA* 2009; 106: 9099–9104.
- 119 Mori T, Saito K, Ohki Y, *et al.* Lack of transient receptor potential vanilloid-1 enhances Th2-biased immune response of the airways in mice receiving intranasal, but not intraperitoneal, sensitization. *Int Arch Allergy Immunol* 2011; 156: 305–312.
- 120 Rehman R, Bhat YA, Panda L, *et al.* TRPV1 inhibition attenuates IL-13 mediated asthma features in mice by reducing airway epithelial injury. *Int Immunopharmacol* 2013; 15: 597–605.
- 121 Delescluse I, Mace H, Adcock JJ. Inhibition of airway hyper-responsiveness by TRPV1 antagonists (SB-705498 and PF-04065463) in the unanaesthetized, ovalbumin-sensitized guinea pig. *Br J Pharmacol* 2012; 166: 1822–1832.
- 122 Baker K, Raemdonck K, Dekkak B, *et al.* Role of the ion channel, transient receptor potential cation channel subfamily V member 1 (TRPV1), in allergic asthma. *Respir Res* 2016; 17: 67.
- 123 Cantero-Recasens G, Gonzalez JR, Fandos C, *et al.* Loss of function of transient receptor potential vanilloid 1 (TRPV1) genetic variant is associated with lower risk of active childhood asthma. *J Biol Chem* 2010; 285: 27532–27535.
- 124 Banner KH, Igney F, Poll C. TRP channels: emerging targets for respiratory disease. *Pharmacol Ther* 2011; 130: 371–384.
- 125 Hox V, Vanoirbeek JA, Alpizar YA, *et al.* Crucial role of transient receptor potential ankyrin 1 and mast cells in induction of nonallergic airway hyperreactivity in mice. *Am J Respir Crit Care Med* 2013; 187: 486–493.
- 126 Devos FC, Boonen B, Alpizar YA, *et al.* Neuro-immune interactions in chemical-induced airway hyperreactivity. *Eur Respir J* 2016; 48: 380–392.
- 127 Gallo V, Dijk FN, Holloway JW, *et al.* TRPA1 gene polymorphisms and childhood asthma. *Pediatr Allergy Immunol* 2017; 28: 191–198.
- 128 Rahman M, Mukherjee S, Sheng W, *et al.* Electrophysiological characterization of voltage-dependent calcium currents and TRPV4 currents in human pulmonary fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 2016; 310: L603–L614.
- 129 Hamanaka K, Jian MY, Townsley MI, *et al.* TRPV4 channels augment macrophage activation and ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2010; 299: L353–L362.
- 130 Groot-Kormelink PJ, Fawcett L, Wright PD, *et al.* Quantitative GPCR and ion channel transcriptomics in primary alveolar macrophages and macrophage surrogates. *BMC Immunol* 2012; 13: 57.
- 131 Dietrich A, Chubakov V, Kalwa H, *et al.* Cation channels of the transient receptor potential superfamily: their role in physiological and pathophysiological processes of smooth muscle cells. *Pharmacol Ther* 2006; 112: 744–760.
- 132 Yang XR, Lin MJ, Sham JS. Physiological functions of transient receptor potential channels in pulmonary arterial smooth muscle cells. *Adv Exp Med Biol* 2010; 661: 109–122.
- 133 Goldenberg NM, Ravindran K, Kuebler WM. TRPV4: physiological role and therapeutic potential in respiratory diseases. *Naunyn Schmiedeberg's Arch Pharmacol* 2015; 388: 421–436.
- 134 Bonvini S, Birrell MA, Dubuis E, *et al.* Activation of TRPV4 triggers ATP release and mast cell dependent contraction of airway smooth muscle (ASM). *Eur Respir J* 2015; 46: Suppl. 59, OA3253.
- 135 Seminario-Vidal L, Okada SF, Sesma JJ, *et al.* Rho signaling regulates pannexin 1-mediated ATP release from airway epithelia. *J Biol Chem* 2011; 286: 26277–26286.
- 136 Mortaz E, Braber S, Nazary M, *et al.* ATP in the pathogenesis of lung emphysema. *Eur J Pharmacol* 2009; 619: 92–96.
- 137 Lommatzsch M, Cicko S, Müller T, *et al.* Extracellular adenosine triphosphate and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010; 181: 928–934.

- 138 Cicko S, Lucattelli M, Müller T, *et al.* Purinergic receptor inhibition prevents the development of smoke-induced lung injury and emphysema. *J Immunol* 2010; 185: 688–697.
- 139 Eltom S, Stevenson CS, Rastrick J, *et al.* P2X7 receptor and caspase 1 activation are central to airway inflammation observed after exposure to tobacco smoke. *PLoS One* 2011; 6: e24097.
- 140 Lucattelli M, Cicko S, Müller T, *et al.* P2X7 receptor signaling in the pathogenesis of smoke-induced lung inflammation and emphysema. *Am J Respir Cell Mol Biol* 2011; 44: 423–429.
- 141 Zhu G, ICGN Investigators, Gulsvik A, *et al.* Association of TRPV4 gene polymorphisms with chronic obstructive pulmonary disease. *Hum Mol Genet* 2009; 18: 2053–2062.
- 142 Birrell M, Bonvini SJ, Baker KE, *et al.* Central role for the TRPV4-ATP-P2X3 axis in sensory nerve activation and the late asthmatic response. *Eur Respir J* 2016; 48: Suppl. 60, PA5069.
- 143 Birrell M, Bonvini SJ, Baker KE, *et al.* The TRPV4 ion channel plays a key role in allergic asthma. *Eur Respir J* 2016; 48: Suppl. 60, OA1792.