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Perspective

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Gene therapy for chronic pain: emerging opportunities in target-rich peripheral nociceptors

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Abstract

With sweeping advances in precision delivery systems and manipulation of the genomes and transcriptomes of various cell types, medical biotechnology offers unprecedented selectivity for and control of a wide variety of biological processes, forging new opportunities for therapeutic interventions. This perspective summarizes state-of-the-art gene therapies enabled by recent innovations, with an emphasis on the expanding universe of molecular targets that govern the activity and function of primary sensory neurons and which might be exploited to effectively treat chronic pain.

Sections

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Introduction

Our brains respond to mechanical, thermal or chemical stimuli as painful when they reach injurious or life-threatening intensities. At the core of pain sensation lies a consortium of ion channels in primary sensory neurons that generate action potentials, which convey signals from the periphery to the CNS.

Under physiological conditions, pain sensation is adaptive but, in a variety of pathological changes, it can take on a life of its own and lead to the generation of persistent pain signals in the absence of noxious stimuli[1,2]. As many as 20–45% of individuals in Europe and the United States are affected by some form of chronic pain[3,4]. Despite its prevalence and major recent basic and translational research advances, the available therapeutic options for chronic pain remain limited. Commonly prescribed non-steroidal anti-inflammatory drugs, glucocorticoids and analgesics such as paracetamol are of limited efficacy[5,6]. Opioid agonists used as first-line therapy for severe pain, while sometimes effective, come with major drawbacks, including dose-limiting adverse effects and addiction[6,7].

Considering the unmet medical needs, the specificity and efficacy of emerging gene therapies offer an attractive alternative for muting chronic pain. Research has revealed a cohort of targets within the electrogenisome — the set of membrane proteins that confer and regulate the electrical excitability of primary sensory neurons in dorsal root ganglia (DRG) and trigeminal ganglia (TG)[8] (Fig. 1). With improving neuronal targeting and precision delivery methods, emerging gene therapies offer unprecedented control over the transfer of therapeutic vectors, with growing prospects for medical use. However, the translation of experimental gene therapies in the treatment of chronic pain comes with substantial challenges imposed by a lack of effective biomarkers and impartial clinical end points as well as emerging biocompatibility and toxicity issues, low stability of genetic material, and potential for off-target action[9–12]. With the recent arrival of precision technologies, new opportunities to overcome these challenges have been recognized, opening prospects for clinical translation.

In this Perspective, we review progress in the pursuit of gene therapy for chronic pain, focusing on ion channels within the nociceptive electrogenisome[8], that is Na^+, K^+, hyperpolarization-activated, cyclic nucleotide-gated (HCN), and transient receptor potential (TRP) channels as well as acid-sensing ion channels (ASICs) and Ca^{2+} channels (Fig. 1a–c). Although traditionally viewed in association with the sensation of touch and pressure, recent studies suggest possible contributions of mechanosensitive PIEZO channels to inflammatory and neuropathic pain, rendering them potential gene therapy targets (Box 1). We consider the expanding universe of therapeutic targets in experimental models and humans, discuss the advantages and limitations of current approaches, and reflect upon challenges and opportunities for future developments. With further improvements in cell targeting and delivery systems, evolving gene therapy methods are anticipated to empower new treatments that might alter the landscape of pain medicine.

Genetic approaches in pain therapy

Recent breakthroughs in targeting and delivery systems and molecular genetics tools have empowered unprecedented access and control over the genomes and transcriptomes of mammalian cells, with implications for nearly all facets of animal and human biology. These developments prompted the revision of the conventional gene therapy model, seeking the substitution of dysfunctional genes[13–15] with new concepts and techniques that allow specific manipulations of the genomes and transcriptomes of affected cells (Fig. 2a–d). For instance, RNA interference (RNAi) shows promise for chronic pain therapy by taking advantage of sequence-specific suppression of gene expression to interfere with protein translation, with several RNAi-based methods approved for clinical use in various non-neuronal disease conditions[16]. Another potentially applicable approach for chronic pain therapy uses antisense oligonucleotides (ASOs), which acts at the transcriptome level to interfere with mRNA processing, leading to the depletion of a protein of interest and suppression of its functions[17]. However, both RNAi and ASO-based interference have limitations in terms of efficiency and cell or tissue specificity[18,19]. Despite major improvements in gene delivery methods, targeting vectors of interest to a specific group of cells (such as primary sensory neurons in patients) remains a challenge, hindering the translation of experimental therapies into clinical use.

Nobel-winning discoveries in gene editing have generated exciting opportunities for a wide range of therapeutic interventions, enabling manipulations at the level of single or small groups of nucleic acids and regulatory elements and thereby offering the prospect of tuning the activity of selected groups of cells, including primary sensory neurons[20,21]. For instance, the CRISPR–Cas9 system has allowed molecular amendments at the DNA level, with major translational prospects (Fig. 2d). Considerable efforts are on the way to improving and broadening CRISPR systems for better efficacy and safety, including tweaking regulatory sites in the genome or CRISPR interference systems using catalytically inactive Cas9 enzyme (dead Cas9 (dCas9)) with a repression domain, which inhibits transcription without altering the DNA sequence in the genome[22]. The precision of CRISPR interference is superior to RNAi and ASO, partly because, unlike interference methods targeting mRNA, dCas9 enables selective manipulations at transcriptional levels. More recent approaches, which take advantage of RNA targeting by adenosine deaminase that acts on RNA (ADAR) or catalytically inactive Cas13, also allow editing of RNA, yielding transient and reversible modulation of protein expression with better safety[23,24]. Like DNA-editing methods, emerging technologies enabling manipulations of epigenetic mechanisms have shown considerable translational potential for clinical use[25].

Most gene therapy systems, including CRISPR, rely on the delivery of transgenes by viral vectors (Table 1), which come with risks of biological incompatibility, genomic stress and unwanted off-target effects[26,27]. Given these challenges, alternative delivery systems have been explored, including the use of stem cells, functionalized liposomes and immunologically neutral nanocarriers[13,28]. Customizing viral capsids, with careful selection of the genome insertion site in the vector and with manipulation of the self-inactivating mechanisms of capsids as well as the use of synthetic delivery systems, is also expected to minimize some of the adverse effects of current gene therapy methods and enhance medical outcomes, including in therapies for chronic pain[11,29].

Voltage-gated Na^+ channels

Of the nine mammalian voltage-gated Na^+ channels ($Na_V1.1$–$Na_V1.9$), $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ are preferentially expressed in primary sensory neurons, where they have essential roles in chronic pain[30]. The most extensively studied and validated as a human pain target is $Na_V1.7$, with gain-of-function causing excruciating pain in inherited erythromelalgia and paroxysmal extreme pain disorder[31,32], whereas $Na_V1.7$ loss-of-function produces total insensitivity to pain, including painless fractures, burns and tooth extractions[33]. After the discovery of mutations in *SCN9A*, which encodes $Na_V1.7$, in these rare

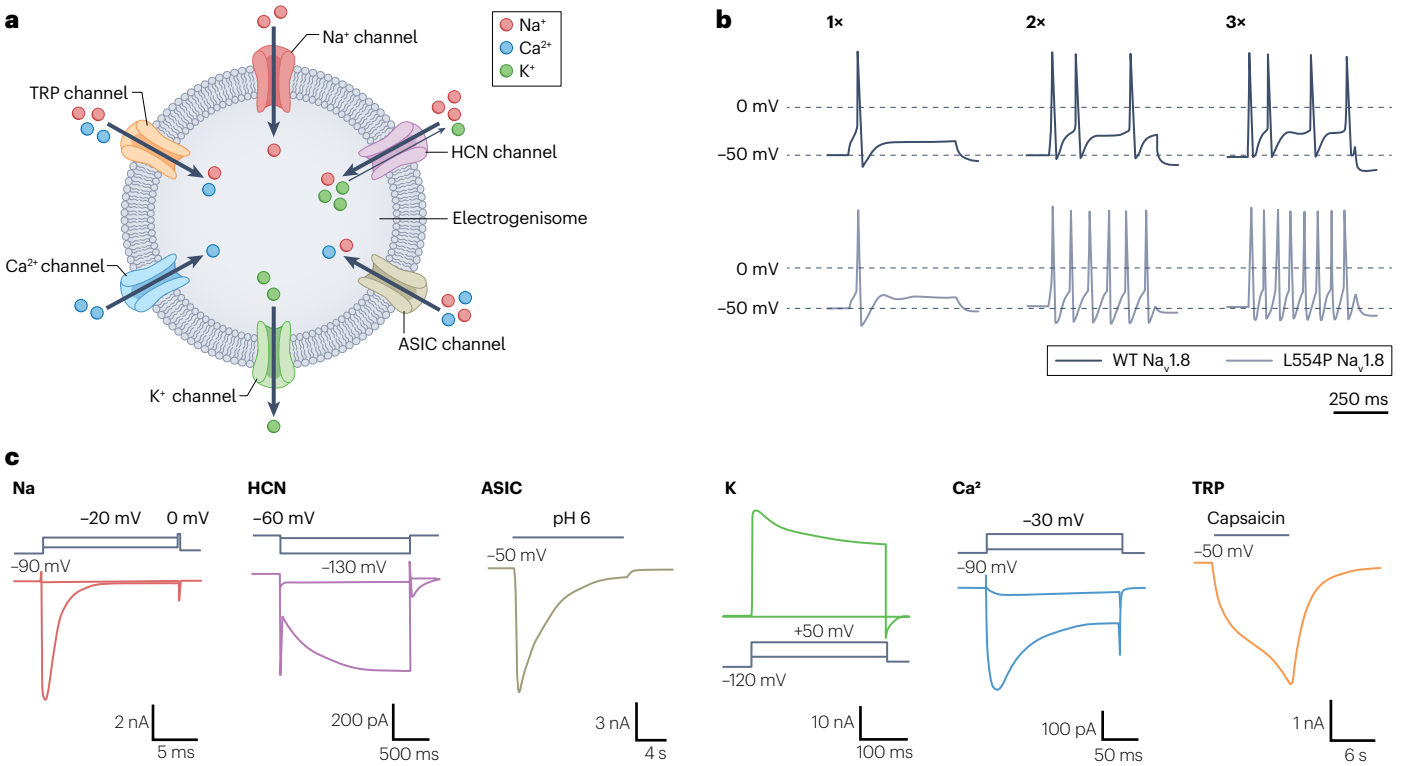


Fig. 1 | Major molecular targets for gene therapy of chronic pain in primary sensory neurons. a, Schematic representation of the principal ion channels in the electrogenisome governing the excitability and generation of action potentials that transmit the pain signal. **b**, As one example, $Na_V1.8$ channels drive repetitive firing of dorsal root ganglia neurons in pain signalling. Shown are recordings of the firing activity evoked by stimuli at 1 ×, 2 × and 3 × current thresholds from dorsal root ganglia neurons of $Na_V1.8$-cre mice lacking endogenous $Na_V1.8$, transfected with either the human wild-type (WT) $Na_V1.8$ channel or the gain-of-function $Na_V1.8$ L554P mutation found in painful neuropathy. **c**, Illustrations of typical currents mediated by hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels, acid-sensing ion channels (ASIC), and Na^+, K^+, Ca^{+2} and transient receptor potential (TRP) channels, which can be modified by various gene therapy approaches described in this Perspective, leading to alterations of the activity of nociceptive neurons and alleviation of chronic pain. Part **b** adapted with permission from ref. [55], PNAS. Part **c** adapted with permission from refs. [55], PNAS;[198], Society for Neuroscience;[199], APS;[200], CC0 1.0 (https://creativecommons.org/publicdomain/zero/1.0/);[201], APS; and[202], APS.

monogenic disorders, *SCNA9* gain-of-function mutations were found in a subpopulation of individuals with the more common painful small-fibre neuropathy[34]. The key role of $Na_V1.7$ in the activity of peripheral nociceptors and the described strong genetic validation have made it an attractive target for gene therapy (Table 2).

Within primary sensory neurons, $Na_V1.7$ acts as a 'threshold channel', amplifying weak inputs and facilitating the generation of action potentials, thereby acting to set the gain on neuronal firing activity like a volume knob[35]. In animal studies, *Scna9* overexpression in sensory neurons has been linked with inflammatory pain, whereas *Scna9* deletion in primary sensory neurons of DRG attenuated inflammatory hyperalgesia[36–38]. Data from *Scna9* knockout (KO) mice suggest that $Na_V1.7$ has a role in hypersensitivity to heat without effects on neuropathic pain and tactile allodynia[39,40], while transfection of gain-of-function mutant $Na_V1.7$ channels into DRG neurons lowers their firing threshold and increases the response of DRG neurons to weak inputs[41]. In line with its role in chronic pain, CRISPR–dCas9-mediated suppression of $Na_V1.7$ in nociceptors attenuated pain responses in mice[42]. In that study, intrathecal infusion of CRISPR–dCas9 adeno-associated virus 9 (AAV9) reduced pain reactions in carrageenan-induced inflammatory models, paclitaxel-induced neuropathic models and BzATP-induced pain models. $Na_V1.7$ channel deficiency also decreased tactile allodynia in neuropathy and reduced thermal hyperalgesia, without changing other sensory functions[42]. Emerging evidence for control of $Na_V1.7$ expression by SUMOylated collapsin response mediator protein 2 (CRMP2) also suggests opportunities for modulation of $Na_V1.7$ activity in sensory neurons[43,44], with CRMP2 emerging as a potential gene therapy target for chronic pain. Taken together, the evidence from preclinical studies establishes the importance of $Na_V1.7$ in inflammatory and neuropathic pain, with the reported ameliorative effects of CRISPR–dCas9 interventions positioning $Na_V1.7$ as a viable gene therapy target (Table 2).

Early studies on DRG neurons also implicated $Na_V1.8$, which functions together with $Na_V1.7$ to produce action potentials in peripheral sensory neurons[45], as a pain target. In DRG neurons, $Na_V1.8$ mediates the bulk of the inward current needed for the rising phase of the action potential[46] and has an essential role in driving firing activity when DRG neurons become depolarized, as occurs in many pathological conditions[45]. $Na_V1.8$ has been implicated in many forms of pain, including inflammatory pain. Accordingly, *Scn8A* KO mice display reduced or delayed carrageenan-induced and NGF-induced thermal hyperalgesia[47]. $Na_V1.8$ enrichment in abdominal nociceptors has also implicated its

Box 1

PIEZO channels of mechanoreceptors

PIEZO channels, widely acknowledged as key players in mechanotransduction, are emerging as potential targets for pain treatment. It is now clear that the nociceptive functions of mechanoreceptors are partly attributable to the activation of the ion channel families PIEZO1 and PIEZO2 (refs. [203,204]). Formed by large homo-trimeric membrane proteins, PIEZO channels are permeant to monovalent (Na^+ and K^+) and divalent (Ca^{2+} and Mg^{2+}) cations[205]. Upon activation mainly by vibration, pressure and stretch, PIEZO channels produce inward currents leading to membrane excitation. In addition, Ca^{2+} influx via PIEZO channels triggers Ca^{2+} signalling mechanisms with a wide variety of effects.

PIEZO channels are expressed in multiple tissues and organs (bladder, lungs, muscles and nerves)[206]. PIEZO2 was shown to be enriched in large dorsal root ganglia neurons mediating diverse mechanoreceptive functions and pain response[204,207]. While PIEZO1 was thought to be absent in primary sensory neurons, more recent studies showed its selective expression in smaller dorsal root ganglia neurons, suggesting the potential importance of PIEZO1 in pain[208]. Genetic knockout mice models and analyses of loss of function in humans imply that PIEZO2 is essential for sensing tactile allodynia as well as gentle touch and proprioception[204,209]. By contrast, PIEZO1 expression in trigeminal ganglia was implicated in migraine pain, as ex vivo hemi-skull preparations showed that PIEZO1 activation caused continuous nociceptive discharge of the meningeal branch of the trigeminal nerve[210]. Recently, the role of PIEZO1 in the inflammatory response has also been shown, with implications for chronic pain[208]. Interestingly, PIEZO1 expression studies suggested that an increase in PIEZO1 level and activity could be associated with a reduction in mechanical pain response[209]. Overall, while this emerging evidence suggests the potential importance of PIEZO1 and PIEZO2 in nociceptive mechanisms and generation of chronic pain, their pertinence as gene therapy targets remains to be elucidated in preclinical models and clinical studies.

role in chronic visceral inflammatory pain[48]. Importantly, the contribution of $Na_V1.8$ in the enhanced spontaneous activity of nociceptors is likely to promote inflammatory pain caused by pro-inflammatory mediators[49,50]. An antisense approach demonstrated that $Na_V1.8$ mediates pain sensitization after chemical irritation of the rat bladder[51]. Attenuation of mechanical allodynia and hyperalgesia with $Na_V1.8$ ASO implies that $Na_V1.8$ has a potential role in neuropathic pain[52,53] (Table 1).

The pathological enrichment of $Na_V1.8$ together with $Na_V1.7$ in painful human neuromas[54] and the tandem operation of these channels in hyperactive DRG neurons[45] implicate $Na_V1.8$ in human pain. The presence of gain-of-function $Na_V1.8$ mutations that produce nociceptor hyperexcitability in painful small-fibre neuropathy adds further evidence that this channel has a role in human pain conditions[55]. Thus, from the gene therapy perspective, $Na_V1.8$ presents an attractive therapeutic target for the amelioration of multiple forms of chronic pain.

$Na_V1.9$ is the third major Na^+ channel implicated in pathological nociception. It produces slow currents over a broad range of membrane potentials, contributing to sustained nociceptive response[56]. Increased expression of $Na_V1.9$ depolarizes the resting potentials of peripheral sensory neurons, lowers their activation threshold and induces spontaneous repetitive firing[57]. $Na_V1.9$ downregulation with ASOs ameliorates mechanical pain in rodent models without adverse effects[58] (Table 2). However, a study of antisense oligodeoxynucleotide (ODN)-mediated knockdown failed to induce antinociceptive effects[59]. Of note, $Na_V1.9$-deficient mice have been reported to show impaired inflammatory pain without neuropathic pain[60], whereas $Na_V1.9$ upregulation has been implicated in trigeminal neuralgia induced by infraorbital nerve constriction[61]. Gain-of-function *SCN11A* mutations have been reported to produce severe pain in humans[62], with dynamic clamp studies showing a direct role of $Na_V1.9$ in increasing the firing of human nociceptors[63]. However, the low expression of $Na_V1.9$ in heterologous systems has hindered the development of methods that target or knockdown $Na_V1.9$. New experimental platforms for research of $Na_V1.9$ are being currently explored and could facilitate the investigation of the utility of gene therapies targeting this channel.

In addition to $Na_V1.7$–$Na_V1.9$, which are constitutively expressed in nociceptors, the $Na_V1.3$ channel, whose expression is downregulated during postnatal development, becomes re-expressed in DRG neurons after peripheral axonal injury[64,65]. Because of the rapid repriming of $Na_V1.3$ channels and the role of $Na_V1.3$ in the subthreshold membrane potential domain, the aberrant presence of $Na_V1.3$ after injury makes neurons hyperexcitable[66,67]. Accordingly, $Na_V1.3$ knockdown in DRG neurons attenuates tactile allodynia in diabetic rat models[68] and mechanical allodynia induced by nerve injury[69]. However, the results of genetic manipulation remain controversial, with some results suggesting that global or DRG-specific $Na_V1.3$ deletion does not alter the pain response[70]. Nevertheless, the re-expression of $Na_V1.3$ channels in DRG neurons following nerve injury, combined with their enrichment in human neuromas[54], highlights the relevance of $Na_V1.3$ as a potential target for therapeutic interference with genetic methods.

Voltage-gated K^+ channels

Human K^+ channels are divided into four subfamilies: voltage-gated K^+ channels (K_V) mediating delayed rectifier and fast inactivating currents; Ca^{2+} activated K^+ channels mediating repolarization and afterhyperpolarization currents; transmembrane-domain inwardly rectifying K^+ channels (Kir); and two-pore domain K^+ channels (K2P) mediating leak currents[71,72]. Members of all four subfamilies are found in mammalian DRG neurons, with a variety of changes in trafficking and expression, current density, activation threshold, and kinetics reported in chronic pain[73,74]. Shaker-related K^+ channel (K_V1) loss-of-function due to an autoimmune response has been reported in neuromyotonia characterized by inflammation and chronic pain[75] and in Morvan syndrome displaying neuromyopathy[76]. By contrast, gain-of-function mutations in *KCNQ2* and *KCNQ3* (encoding $K_V7.2$ and $K_V7.3$)[77,78] stabilized the resting potential of DRG neurons and attenuated chronic pain by countering DRG hyperexcitability caused by the *SCNA9* gain-of-function mutations discussed above[79]. These latter observations suggest that gene therapy approaches that boost $K_V7.2$ and $K_V7.3$ expression within DRG neurons might provide effective means for the alleviation of chronic pain. In addition, multiple single-nucleotide polymorphisms of *KCNS1* (encoding $K_V9.1$) that predispose to chronic pain have been reported[80]. Given the ubiquitous presence of K^+ channels in neurons and glial cells that are important for neuronal excitability and action potential

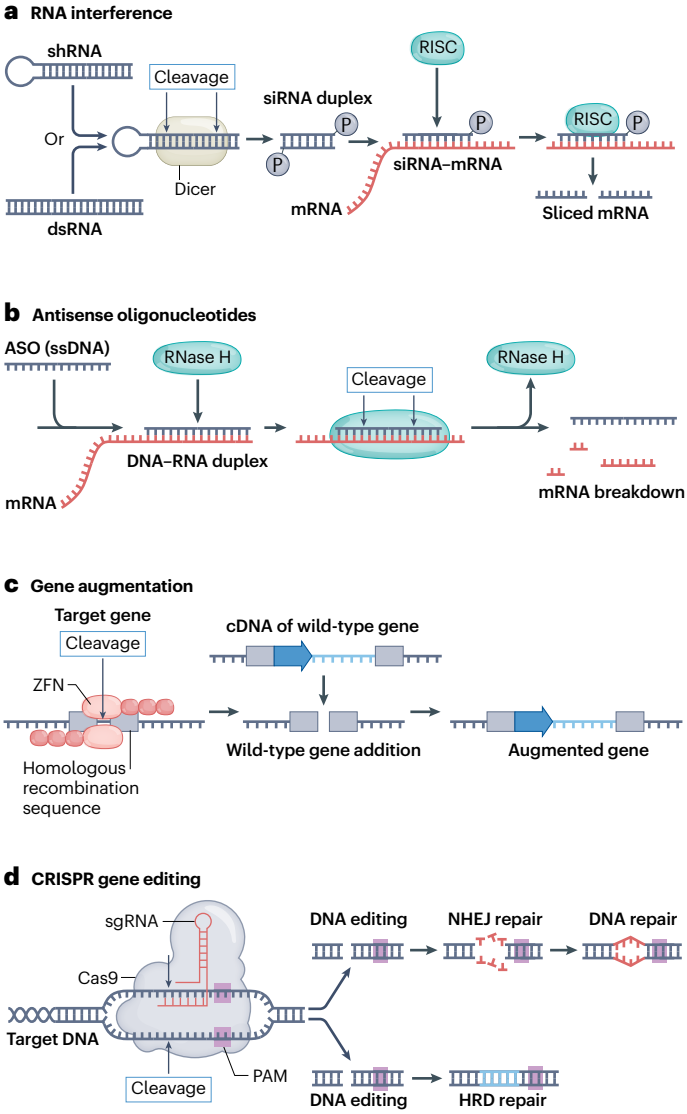


Fig. 2 | Illustration of major gene therapy approaches used in preclinical models of chronic pain. a, RNA interference forms small RNA double strands to target specific genes and residues of protein expression in neurons. After cleavage of short hairpin RNA (shRNA) or double-stranded RNA (dsRNA), small interfering RNA (siRNA) is formed, which reacts with messenger RNA (mRNA) to provide a platform for the RNA-induced silencing complex (RISC) to engage with in order to slice and inactivate the mRNA and block protein translation. **b**, Antisense oligonucleotides (ASO) hybridize with specific nucleotide sequences of targeted mRNA or micro RNA in neurons. ASO identifies and hybridizes with mRNA in a cDNA–RNA duplex, attracting RNase H enzyme, which cleaves and breaks down mRNA, inhibiting protein translation. **c**, Gene augmentation boosts gene expression to compensate for functional deficiency or to counter excessive activity of neurons. After cleavage of the target gene by zinc finger nuclease (ZFN), cDNA of the wild-type gene gets inserted in the host genome (affected by a loss-of-function mutation) to encode a protein with functional enhancement. **d**, The CRISPR gene-editing system modifies specific target genes in the genome, in RNA or an organism to alter their activity in neurons. By targeting Cas9 nuclease to a specific segment of the genome with single guide RNA (sgRNA), contingent on protospacer adjacent motif (PAM) and matching sequences, the target DNA is cut at a specific site. This is followed by editing of the DNA and non-homologous end joining (NHEJ) or homologous recombination deficiency (HRD) repair, which can enable the addition or removal of DNA fragments, leading to changes in protein synthesis with predictable functional alterations. ssDNA, single-stranded DNA.

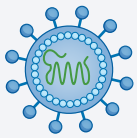
generation[72,81], it is not surprising that K^+ channels have been implicated in multiple pain conditions and are of major relevance as a target to emerging gene therapies.

K_V1 channels could represent a promising gene therapy target. The unique coupling of S4 and S5 domains in the $K_V1.1$ subunit bestows its mechanoreceptive properties[82], with genetic and pharmacological blockade inducing mechanical hypersensitivity without pain[83]. $K_V1.2$, the main subunit partner of $K_V1.1$, has been implicated in chronic pain in several rodent studies. For instance, peripheral nerve injury led to decreases in $K_V1.2$ mRNA and protein levels in DRG neurons[84], effects that were mediated by upregulation of an antisense long non-coding RNA that lowered $K_V1.2$ expression and resulted in membrane hyperexcitability and sensitization[85] (Table 2). Inhibition of long non-coding RNA upregulation using antisense RNA in a spinal nerve ligation model attenuated the pain response and countered the injury-related downregulation of $K_V1.2$ expression[85]. Furthermore, $K_V1.2$ over-expression using AAV5 full-length $K_V1.2$ sense RNA in the spinal nerve ligation model increased $K_V1.2$ mRNA and protein levels and mitigated mechanical allodynia as well as thermal and cold hyperalgesia[85]. Importantly, $K_V1.2$ antisense RNA fragment or full-length $K_V1.2$ antisense RNA did not alter basal nociception, capsaicin-induced pain or locomotor function[84,85]. By contrast, $K_V1.2$ knockdown by small interfering RNA (siRNA) induced mechanical and thermal hypersensitivity[86]. Nociceptive mechanisms related to the $K_V1.2$ subunit could also be targeted using microRNA (miRNA) because $K_V1.2$ function is controlled by non-coding miR-137 (ref. [86]). Indeed, targeting and inhibition of $K_V1.2$ using an miR-137 agomir alleviated mechanical allodynia and thermal hyperalgesia in rats subject to chronic constriction injury (CCI)[86]. Finally, epigenetic silencing of *Kcna2* (encoding $K_V1.2$) by G9a (histone-lysine *N*-methyltransferase 2) has proven feasible, reversing the sensitization of peripheral afferents involved in generating pain in a nerve injury model[87].

In addition to the direct targeting of K_V1 channel activity to modulate pain, the involvement of shaker-related channels can also be modulated. Alterations in adhesion protein CASPR2, which anchors $K_V1.1$, $K_V1.2$ and $K_V1.6$ subunits at juxta-paranodes of primary sensory axons, can regulate the function of K_V1 channels in myelinated axons[88]. Induction of pain-related hypersensitivity in mice without neural injury by human CASPR2 auto-antibodies[89] suggests that generic targeting of this regulatory mechanism might be relevant to pain therapy. However, it is interesting to note that the contribution of various K_V1 subunits to acute and chronic pain could differ, as reported for the $K_V1.6$ subunit[90], suggesting that remodelling of K_V1 channels and their subunit content depends on the specific pain condition. Overall, it is reasonable to expect that genetic interventions with shaker-related channels might provide a targeted and specific means for amelioration of chronic pain.

$K_V2.1$ is also of major relevance to chronic pain (Table 2). Dysfunction of this K_V2 channel subunit in DRG neurons caused by injury leads to the enhanced firing activity of nociceptors in response to sustained inputs[91] because the slow kinetics and high activation threshold of $K_V2.1$ have a stabilizing role during prolonged firing. Importantly, $K_V2.1$ is regulated by members of the K_V5, K_V6, K_V8 and

Table 1 | Summary of major characteristics of common viral vectors used in gene therapy of chronic pain

Vector	Size (nm)	Genome	Capacity (kB)	Transduction	Transduction efficiency	Immunogenicity	Integration	Expression	Biosafety
AV 	~90–100	dsDNA	~8–36	D & ND	High	High	Non-integrating	Days to weeks	BSL-2
AAV 	~25	ssDNA	~4.7	D & ND	Moderate	Low to moderate	Integrating	>1 year	BSL-1
LV 	~80–100	ssRNA	~8	D & ND	Moderate	Moderate to high	Integrating	Lifelong	BSL-2
HSV1 	~80–100	dsDNA	~40	dsDNA	Moderate	Low to moderate	Non-integrating	>1 year	BSL-2

AAV, adeno-associated virus; AV, adenovirus; BSL, biosafety level; D, dividing; dsDNA, double-stranded DNA; HSV1, herpes simplex virus 1; LV, lentivirus; ND, non-dividing; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA.

K_V9 channel subfamilies, that is, the 'silent subunits' (K_VS)[92]. Given that K_VS promote hyperpolarization and accelerate the inactivation of $K_V2.1$-containing channels[93], genetic manipulations of K_VS expression might be useful for chronic pain management. Accordingly, $K_V9.1$ downregulation following axotomy or siRNA knockdown in rats reduced $K_V2.1$ activity and enhanced neuronal excitability, leading to augmentation of the pain response[73,94]. Likewise, deletion of *Kcns1* promoted the development of neuropathic pain[95], while siRNA-mediated inhibition of $K_V9.1$ in rats led to neuropathic pain[94]. Finally, loss-of-function mutations in KCNG4 (encoding $K_V6.4$) were shown to promote the excitability of TG neurons during human migraine attacks[96] and pain sensation during childbirth[97], with these effects attributed to dysfunction of $K_V2.1$. The available data support the need for additional studies of K_VS targeting as a potential strategy for adjusting $K_V2.1$ activity to control chronic pain.

K^+ channels from the K2P subfamily implicated in pathological pain include TRAAK, TREK1 and TREK2, which act as mechanosensors and thermosensors, with their deletion in DRG neurons enhancing sensitivity to mechanical stress, heat and oxaliplatin-induced cold[98,99]. These sensitivity alterations seem to be mediated by reduced rheobase and increased excitability of primary sensory neurons[100] as decreased mRNA of another K2P channel (TRESK, also known as KCNK18, caused by sciatic nerve transection) lowered the pain threshold to mechanical stimuli[101]. The Kir subfamily of K^+ channels also contributes to chronic pain via altering microglial response to inflammation[102,103]. Finally, impairments in buffering extracellular K^+ during intense neuronal firing in *Kcnj10* KO mice lacking Kir4 suggest a role for these channels in regulating membrane potentials through K^+ uptake via satellite glia[104]. Interestingly, there is evidence suggesting that members of the Kir3 subfamily (also known as GIRK1–4 proteins) can contribute to spinal and general analgesia, in part via coupling to G proteins linked to opioid receptors and the related antinociceptive response[105]. While the results of functional studies support the involvement of Kir and K2P channels in the generation of pain responses, their suitability as a target for gene therapy of chronic pain remains to be shown.

Transient receptor potential channels

TRP channels are grouped into six subfamilies on the basis of sequence homology: TRPA, TRPV, TRPM, TRPC, TRPP and TRPML, which are expressed along the entire neural axis[106]. Since the discovery of TRP channels as capsaicin and heat receptors[107], major progress has been made in elucidating their structure and functions, with several endogenous and exogenous ligands identified[108,109]. Genetic mutations in TRP channels are associated with several channelopathies affecting sensory functions[110]. One of the best characterized, rs10166942, is linked with lowered *TRPM8* expression and reduced migraine incidence[111]. A *TRPM8* missense mutation (p.Arg30Gln) is found in individuals with familial trigeminal neuralgia and causes enhancement of TRP channel activation, thereby increasing TRP current amplitude and intracellular Ca^{2+}

transients[112]. TRPM8 response to heat is fine-tuned by phosphorylation at several sites and regulated through interactions with dendrotoxin-sensitive (DTX-I) K_V1 channels — mechanisms that can have a notable role in gating thermoreception in primary sensory neurons and the related pain response[113,114]. Furthermore, *TRPV1* mutations are found in individuals with severe, long-lasting pain following corneal refractory surgery, which severs the axons of TG neurons, suggesting that TRPV1 has a role in chronic postoperative pain[115]. As evident from the emerging clinical reports and from preclinical studies, dysfunctional TRP channels contribute to a variety of pain-related conditions; therefore, TRP channels might also be tractable targets for gene therapy of chronic pain (Table 2).

In addition to sensing peripheral noxious, chemical and thermal stimuli, TRP channels are involved in pain transmission at the central terminals of primary sensory neurons. Activation of TRPV1 and TRPA1 in brain slices or by intrathecal infusion of capsaicin or mustard oil in the brain increased the release of glutamate and neuropeptides implicated in the pain response and sensitization of primary afferents[116,117], with both channels contributing to burning, itching, piercing, pricking and stinging sensations. TRPV1 integration of these multiple painful stimuli is particularly well studied. For example, compensatory overexpression of TRPV1 in response to nerve injury stimulated the release of neuropeptides associated with pain and led to hyperactivity of nociceptive neurons[118,119]. Deletion studies of TRP genes also suggest that TRP channels have a role in various aspects of nociception, with relevance to chronic pain. For instance, *Trpv4* KO mice displayed attenuated pain-induced behaviour in several tests, including the visceral pain response[120]. Finally, pharmacological blockade of TRPC5 prevented mechanical hypersensitivity and reduced tactile nociception as well as pain sensation induced by skin incision, chemotherapy or complete Freund adjuvant (CFA) injection[121,122].

In animal studies, the role of TRPV1 in nociception has been validated by genetic deletion, depletion of TRPV1 transcripts with siRNA, antibody-induced blockade of the TRPV1 channel and its inhibition with antagonists[123–126]. Intrathecal administration of TRPV1 siRNA in mice attenuated capsaicin-induced visceral and neuropathic pain, whereas administration of TRPV1 ASOs reversed mechanical hypersensitivity in spinal nerve-ligated rats[127,128]. These results are of notable interest given that the antinociceptive effects induced by systemic pharmacological inhibitors of TRPV1 have major adverse effects. In another study, injection of AAV6 encoding a TRPV1 interfering peptide aptamer (a short DNA sequence that specifically binds TRPV1, encompassing residues 735–772) into DRG caused a reduction in Ca^{2+} currents and attenuation of capsaicin-induced cytoplasmic Ca^{2+} transients in sensory neurons in a rat model of neuropathic pain[129]. The same vector applied to rat DRG neurons resulted in attenuation of the symptoms of traumatic nerve injury[129]. With improved delivery vectors and precise targeting, similar genetic interventions might provide effective means for the treatment of chronic pain, especially in conditions associated with heat hypersensitivity and neuropathy.

The gene therapy potential of TRPV1 targeting was also shown in a rat model of orthodontic pain using short hairpin RNA (shRNA)[130]. In this study, TRPV1 had a key role in the intensification of orofacial pain, with TRPV1 protein and mRNA levels enhanced by tooth movement. Delivery of shRNA lowered the expression of TRPV1 and eased the pain response, suggesting the utility of *TRPV1* shRNA as a gene therapy for orthodontic pain[130]. Lentiviral targeting to TG neurons using an anti-p75 neurotrophin receptor (NTR) antibody achieved retro-axonal delivery of *TRPV1* gene therapy[126]. After the mitigation of capsaicin response in cultured neurons was demonstrated, suppression of TRPV1 activity in the TG of rats resulted in an antinociceptive effect. Another report in a mouse model of inflammatory hyperalgesia showcased the utility of CRISPR–Cas9 editing for inhibition of TRPV1 phosphorylation by protein kinase C (PKC)[131]. Without blocking physiological TRPV1 functions, CRISPR–Cas9 editing of PKC phosphorylation residue S801 of TRPV1 reduced the pain caused by masseter muscle inflammation in these mice[131]. Overall, the depletion of TRPV1 channels in TG and DRG nociceptors with shRNA or the editing of TRPV1 regulatory sites by CRISPR–Cas9 present viable therapeutic gene intervention options for mechanical allodynia, inflammatory pain and hypersensitivity caused by a traumatic injury. These encouraging results with gene therapy of TRP receptors underscore further research and their prospective use as targets for gene therapy.

Acid-sensing ion channels

ASICs are H^+-gated channels expressed in CNS and peripheral sensory neurons that function as sensors of pH changes caused by ischaemia, inflammation, trauma and other pathological conditions[132]. ASIC subunits include six members (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4) encoded by four genes[133,134]. A functional ASIC consists of ASIC1a, ASIC2a and ASIC3 subunits forming homotrimers or heterotrimers[135,136]. ASIC2b does not form H^+-gated channels but can influence the properties of ASICs produced by the other subunits[137,138], whereas ASIC4 regulates the trafficking of functional ASIC channels[138]. Because of differential proton sensitivity, activation and inactivation kinetics and pharmacological profiles[139], expression differences and functional

Table 2 | Major pain-related gene therapy targets in the electrogenisome of primary sensory neurons

Gene therapy target	Neurophysiological processes mediated	Gene therapy approaches used in preclinical studies
$Na_V1.7$	Membrane excitability, action potential threshold	Knockdown
$Na_V1.8$	Membrane excitability, sustained action potential firing	Knockdown
$Na_V1.9$	Membrane excitability, action potential firing	Knockdown
$K_V1.2$	Membrane excitability, synaptic transmitter release	Augmentation
$K_V2.1$	Membrane excitability, prolonged action potential firing	Augmentation
TRPV1	Membrane excitability, release of pain mediators	Knockdown
ASIC3	Membrane potential, action potential firing	Knockdown
$Ca_V1.2$	Membrane excitability, neurotransmitter release	Knockdown
$Ca_V2.2$	Membrane excitability, neurotransmitter release	Knockdown
$Ca_V3.2$	Membrane excitability	Knockdown

Knockdown achieved using approaches such as CRISPR–Cas9, small interfering RNA and antisense oligonucleotides. ASIC3, acid-sensing ion channel 3; Ca_V, voltage-gated Ca^{2+} channels; K_V, voltage-gated K^+ channels; Na_V, voltage-gated Na^+ channels; TRPV1, transient receptor potential cation channel subfamily V member 1.

changes in various ASIC subunits can have wide-ranging effects on pain responses.

Functional and behavioural characterization of *Asic1*, *Asic2*, *Asic3* and *Asic4* KO mice showed differences in pain sensing not only in comparison with wild-type mice but also between the various KO mice lines. While abundant data supported the role of ASICs in the nociceptive response, residual H^+-activated currents in DRG neurons of the KO models complicated the interpretation of the results[135,140]. Immunohistochemical studies showed ample presence of ASIC1a, ASIC1b, ASIC2a and ASIC2 in the CNS of rodents[134,139]. ASIC1a appears as the most ubiquitous and important functional subunit in nociceptors, as evident from the results of biochemical and pharmacological tests[139,141]. While reports of other ASIC1 and ASIC2 subunit-containing nociceptors support their potential involvement in pathological pain, functional studies show a major redundancy of various ASIC1 and ASIC2 subunits in nociceptors, calling for in-depth analysis with the characterization of the specific role of each subunit. The functional overlap and complex interactions of ASIC1 and ASIC2 in nociceptors also complicate the analysis of their translational relevance to various aspects of pathological pain and their targeting with gene therapy.

Unlike ASIC1 and ASIC2, which are expressed uniformly in all neurons, ASIC3 is enriched in the peripheral nervous system (PNS) and is the most prevalent ASIC subunit in DRG[142], where it has a key role in inflammatory pain. In DRG neurons, *Asic3* expression increases after nerve injury, while *Asic3* deletion shortens and attenuates mechanical and thermal hyperalgesia in preclinical studies of neuropathic pain[143,144] (Table 2). Analysis of orthodontic pain in rats showed that *Asic3* expression is increased in TG neurons in the orthodontic force model, with *Asic3* silencing by shRNA alleviating mechanical hyperalgesia[145]. ASIC3 suppression by intrathecal administration of siRNA prevented CFA-induced heat hyperalgesia and flinching caused by acidified capsaicin, serotonin or formalin[142]. In addition to mitigating inflammation and the related nociceptive response, inhibition of ASIC3 eliminated secondary mechanical hyperalgesia of the paw of mice following joint and muscle inflammation.

To verify the specific role of ASIC3 in pathological pain, its functionality in *Asic3* KO mice was rescued by delivery of a full cDNA of the ASIC3 channel with a recombinant herpes simplex virus (HSV)[146,147]. In the same vein, the re-expression of ASIC3 in afferents of the gastrocnemius muscle of ASIC3 KO mice with a recombinant HSV vector restored mechanical hyperalgesia[148], implying that, in secondary hyperalgesia, muscle afferents might be the primary site of ASIC3 effects. Another study used artificial miRNAs (miR-ASIC3) directed against mouse *Asic3* to validate its potential relevance to chronic pain[146]. In CHO-K1 cells transfected with *Asic3* cDNA, miR-ASIC3 inhibited the expression of ASIC3 and lowered acidic H^+-evoked currents without altering the functions of co-transfected ASIC1a subunits[146]. Then, in the carrageenan-induced inflammation model, miRNA-ASIC3 delivery with HSV reduced paw and gastrocnemius muscle mechanical hyperalgesia and suppressed ASIC3 mRNA and protein expression in DRG neurons and gastrocnemius muscle. These findings agree with the reported pharmacological effects of ASIC antagonists, which reduced hyperalgesia associated with muscle inflammation evoked by carrageenan or exercise[132,141]. Together, these studies imply that similar downregulation of ASIC3 in human afferents innervating muscle might counter hyperalgesia and the inflammatory pain response.

Overall, unlike the ASIC1 and ASIC2 subunits, the ASIC3 subunit emerges as a promising gene therapy target with major relevance to neuropathic and inflammatory pain and other pain-related conditions concerned with changes in tissue pH, including ischaemia and cancer pain[132,139]. Nonetheless, clinical translation of experimental observation in animal models has been obstructed by the scarcity of mechanistic data and conflicting reports[149]. Additionally, it remains unclear how the results of ASIC3 studies in rodents, where it is predominantly expressed in the PNS, relate to humans, where ASIC3 subunits are more ubiquitously distributed throughout the CNS and PNS[132,150].

Hyperpolarization-activated, cyclic nucleotide-gated channels

Four members of the HCN channel family (HCN1–HCN4) belong to the superfamily of K_V and cyclic nucleotide-gated channels activated by hyperpolarization and cAMP[151,152]. Upon activation, HCN channels produce a hyperpolarization-activated current that drives the membrane towards the resting potential. Indeed, HCN channels have selective permeability for Na^+ and K^+ and adopt an open state at rest, which makes them an important regulator of membrane excitability. Dysfunction of HCN channels has been reported in various neurological disorders, including epilepsy, cerebellar ataxia, Parkinson disease and Alzheimer disease[153–155]. Studies of sensory neurons using pharmacological and genetic manipulations suggest that HCN1, HCN2 and HCN4 can contribute to chronic pain, whereas the involvement of HCN3 in pain remains controversial[153].

Comparison of HCN channel expression in different groups of DRG neurons showed a notable heterogeneity, with HCN1 dominating in large mechanoreceptors, while HCN2 was prevalent in medium and small neurons mediating nociception, chemoreception and thermoreception[156,157]. By contrast, HCN3 and HCN4 displayed low and indiscriminate expression in all DRG neurons[157,158]. Because HCN2 controls the firing of pain-sensing primary sensory neurons[159], it is viewed as a potential therapeutic target in neuropathic and inflammatory pain, but the results of preclinical studies vary depending on the model and experimental design. For instance, in CFA-induced inflammatory pain, deletion of *Hcn2* (encoding HCN2) in nociceptors prevented mechanical but not thermal hyperalgesia[160], whereas, in another study, *Hcn2* KO prevented thermal but not mechanical hyperalgesia in prostaglandin E2 (PGE2)-induced inflammatory pain[161]. Importantly, *Hcn2* deletion prevented both thermal and mechanical hyperalgesia in neuropathic pain induced by CCI of the sciatic nerve[161]. Furthermore, the loss of HCN2 in primary afferents attenuated the formalin-induced pain response in rodents (licking, biting, paw lifting), which is thought to be due to the release of inflammatory mediators[161]. From the gene therapy perspective, differential expression of HCN1 and HCN2 channels in different groups of primary sensory neurons presents an opportunity for targeted modifications of their functions for the management of various forms of pathological pain. Molecular profiling of primary sensory neurons demonstrating several distinct types of cell[162] support the prospect of their precision targeting to achieve functional alterations with specific therapeutic outcomes.

Difficulties in translating basic research of HCN channels in the context of pain therapy are largely due to the dual electro-chemical nature of HCN channel activation and the complex interplay between various HCN channel subtypes. For instance, in small nociceptors, deletion of *Hcn2* abolished the voltage shift of the hyperpolarization-activated current following cAMP elevation, whereas deletion of *Hcn3* did not alter this voltage shift[163], in agreement with the higher sensitivity of HCN2 to cAMP. By contrast, while genetic deletion of *Hcn3* had little effect on evoked firing in small DRG neurons, it enhanced the excitability of medium-sized DRG neurons, implying a potential role for HCN3 in specific aspects of pathological pain[153,163]. In the context of chronic

pain, deficiency in HCN2 function attenuated diabetes-associated mechanical allodynia and prevented the activation of secondary sensory neurons in the spinal cord in a diabetic pain model[164]. The same study showed an increase in intracellular cAMP in secondary sensory neurons, implying that cAMP might regulate the level of pain by tuning HCN2 activity. It is interesting to note that the deletion of HCN2 from nociceptive neurons abolishes heat-evoked inflammatory pain and all aspects of neuropathic pain in rats, while leaving acute pain sensation intact[159,161]. Thus, inflammatory and neuropathic pain have much in common and might be silenced by gene therapies targeting HCN2.

Despite the presence of HCN3 in all groups of DRG neurons and its alleged role in the excitability of medium-sized sensory cells, HCN3 deletion had little or no impact on inflammatory and neuropathic pain in functional tests[163]. Indeed, HCN3-deficient mice had no change in the threshold of pain response to heat or mechanical stimuli, with nociception also unaltered by inflammation[163]. Nonetheless, levels of mechanical allodynia and thermal hyperalgesia in nerve-injured *Hcn3* KO mice were similar to those in wild-type mice, whereas *Hcn3* KO mice had reduced mechanical hyperalgesia in response to pinprick[163]. In addition to the differential effects to various nociceptive stimuli, HCN2 and HCN3 alterations also display differential effects on various phases of pain response. For instance, unlike HCN3 deletion, HCN2 deletion did not affect the initial rapid phase of the nociceptive reaction to formalin injection (licking and biting of the injected paw) but lowered the slowly developing inflammatory pain response[161].

In summary, the results of functional studies of HCN channels in primary sensory neurons advocate their relevance as gene therapy targets for the treatment of several aspects of chronic pain. However, their indiscriminate expression in primary sensory neurons with functional redundancy among various members sets major challenges for the selective manipulation of pain response by targeting HCN channels. The intricacies of the biology of HCN channels along with the complex neurobehavioural phenotypes of preclinical research models have undoubtedly contributed to the slow progress in translational studies of these channels as targets for gene therapy of chronic pain.

Voltage-gated Ca^{2+} channels

Ca^{2+} channels are divided on the basis of their activation voltage into high-voltage-activated (HVA) channels, which comprise L-type ($Ca_V1.1$– $Ca_V1.4$), P/Q-type ($Ca_V2.1$), N-type ($Ca_V2.2$) and R-type ($Ca_V2.3$) channels, and the low-voltage-activated (LVA) T-type ($Ca_V3.1$–$Ca_V3.3$) channels[165]. The most abundantly expressed Ca^{2+} channels in DRG neurons are N-type and T-type channels, followed by $Ca_V1.2$ L-type channels[166,167]. R-type channels are absent in DRG neurons while P/Q-type channels are expressed in trace amounts, regulating neurotransmitter release from C and δ fibres[168].

The nociceptive role of Ca^{2+} channels involves two mechanisms: (1) membrane depolarization and release of excitatory transmitters, and (2) regulation of Ca^{2+}-activated K^+ currents that control the membrane potential and firing activity of neurons[1]. Most of the data supporting the role of Ca^{2+} channels in pathological pain comes from pharmacological studies[1,168], with recent genetic evidence advocating Ca^{2+} channel involvement in inflammatory and neuropathic pain. For instance, $Ca_V1.2$ knockdown in dorsal horn neurons using siRNA reversed nerve injury-related mechanical hypersensitivity[169] (Table 2). CCI of the sciatic nerve causes mechanical allodynia, partly attributed to the upregulation of the α2δ-1 accessory subunit of $Ca_V1.2$ in nociceptors[170]. Anti-$Ca_V1.2$ siRNA or $Ca_V1.2$ knockout in the dorsal horn neurons eliminated mechanical allodynia caused by damage of the sciatic nerve, suggesting that gene therapy has potential utility for countering nociceptor sensitization and mechanical allodynia involving L-type channels. Interestingly, increased $Ca_V1.2$ in the spinal cord after spinal nerve ligation, which correlated with hypersensitivity, was associated with the downregulation of $Ca_V1.2$ and $Ca_V1.3$ in DRG neurons[171], implying complex regulation of L-type channel expression and function.

Reduced inflammatory and neuropathic pain in N-type channel-deficient mice supports the role of N-type channels in pathological pain[172], with experimental axotomy causing a significant reduction in N-type channel expression in DRG neurons[173,174]. Targeting CRMP2, which is known to enhance synaptic transmission, showed the therapeutic utility of genetic manipulations of $Ca_V2.2$ in pathological pain, similar to $Na_V1.7$ (ref. [175]). Using a short peptide designated Ca^{2+} channel-binding domain 3 (CBD3) to disrupt CRMP2 interactions with $Ca_V2.2$ led to inhibition of N-type current with an antinociceptive outcome[176]. AAV overexpression of large conductance (BK) Ca^{2+}-activated K^+ channels, known to compete with $Ca_V2.2$ for α2δ-1 subunits, countered the upregulation of N-type channels in DRG neurons[177]. This resulted in a reduction of N-type channel expression and function, with intrathecal delivery of the N-terminus region of the BK channel producing lasting analgesia in mouse models of inflammatory and neuropathic pain. Together, these observations suggest that gene therapy targeting N-type channels in primary sensory neurons might pre-empt, and potentially reverse, neuropathic and inflammatory pain (Table 2).

While counterintuitive, the blockade of HVA Ca^{2+} channel currents can also increase the excitability of neurons via a reduction in Ca^{2+}-activated K^+ currents. A genetically encoded auxiliary $Ca_V\beta$ subunit-targeted nanobody (nb.F3) fused to the catalytic HECT domain of the E3 ubiquitin ligase Nedd4L (Ca_V-aβlator) was used to inhibit the activity of HVA Ca^{2+} channel currents[178]. Upon delivery by AAV9 into the hind paw of mice, Ca_V-aβlator expression in a subset of DRG neurons lowered HVA Ca^{2+} channel currents and enhanced spontaneous inhibitory postsynaptic currents in dorsal horn sensory neurons. In addition to providing proof-of-concept for the delivery of Ca_V-aβlator by AAV with blockade of Ca^{2+} channel currents in vivo, the report suggests that this approach can lead to alleviation of pain, which results not from the reduction in excitatory inputs to dorsal horn neurons but from changes in activity in spinal cord circuitry[179]. Of note, the antinociceptive effects of Ca_V-aβlator expression in DRG neurons after nerve injury extend to all HVA channels[178,180]. Along with the described antinociceptive effects induced by AAV-targeted CBD3 peptide[176], these results uncover notable prospects of selective modulation of specific HVA Ca^{2+} channel subtypes and a more versatile approach for interference with HVA channels in DRG neurons with an antinociceptive outcome (Table 2).

Unlike HVA N-type channels, Ca^{2+} influx via LVA T-type channels is activated by weak depolarization, contributing to the nociceptive response primarily by tuning the near-threshold excitability and neuronal response to weak inputs[1,168]. T-type channels also regulate the neuronal output of primary sensory neurons by driving them in rebound bursting mode, which has also been implicated in pain responses[181]. In addition, some evidence suggests that T-type channels can regulate nociceptive responses through interactions with presynaptic syntaxin 1A and SNAP25 proteins[182]. In line with their involvement in regulating pain response, the density of T-type currents in DRG neurons increased after peripheral nerve injury in rat models of diabetic neuropathy and spinal cord injury[183,184]. While there are no known human mutations of *CACNA1H* (encoding $Ca_V3.2$) that produce a painful phenotype, conditional knockout of this T-type channel attenuated mechanical allodynia linked to neuropathic pain in mice[185]. *CACNA1H*

variants found in individuals with trigeminal neuropathy suggested changes in activation and inactivation of T-type channels that could result in TG neuron hyperexcitability, implying that T-type channels have an important role in trigeminal chronic pain[186]. Another gene therapy approach is suggested by the observation that disruption of $Ca_V3.2$ ubiquitination by the USP5 enzyme can cause allodynia linked to neuropathic pain in a mouse model, whereas constitutive ubiquitination counters mechanical hypersensitivity in inflammatory and neuropathic pain[187]. Antinociceptive effects were also achieved with intrathecal $Ca_V3.2$ ASO, which caused an ~80% decrease in T-type currents in DRG neurons and attenuation of nociceptive responses in naive and neuropathic rats[188], whereas similar interventions with $Ca_V3.1$ or $Ca_V3.3$ produced no effects[188].

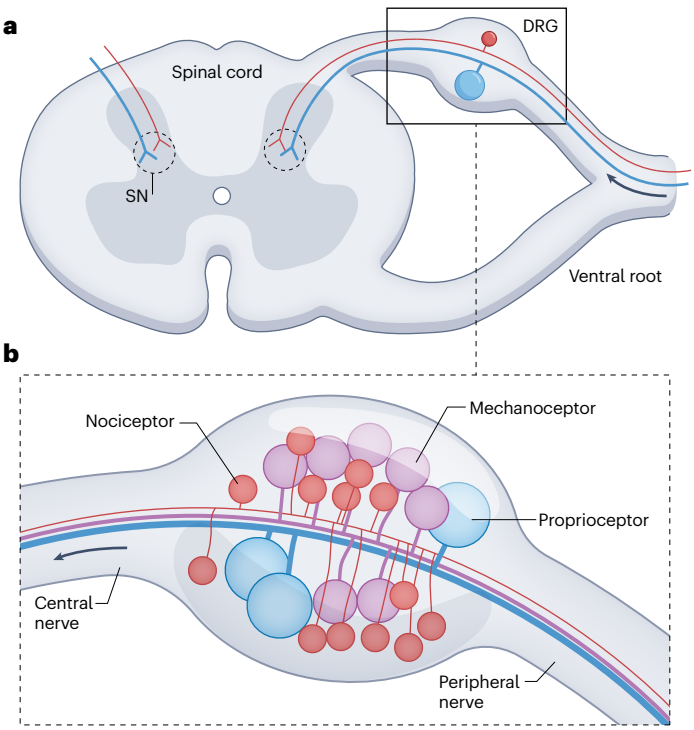
Taken together, the results of the manipulation of Ca^{2+} channels support their relevance to the generation of chronic pain and utility as a gene therapy target.

Future directions and prospects

Since the first proof-of-concept studies in the 1980s, major strides have been made in basic research and clinical translation related to gene therapy, delivering multiple life-changing treatments[11,13]. Over the past decade, invigorated by the success of the Human Genome Project and technological innovations, the focus of gene therapy has shifted from the replacement of faulty genes to genome and transcriptome editing as well as fine-tuning of gene activity and functions. Newly arriving tools and methods are driving this shift by empowering unmatched precision and control over the coding and non-coding genome with increasingly controllable functional outcomes[11,20]. With over 800 gene and cell therapy programmes under development and more than a dozen approved by the European Medicines Agency and the Food and Drug Administration, gene therapy has unveiled major opportunities for personalized medicine for a variety of diseases[189,190]. For more than 1.5 billion people worldwide affected by chronic pain, these developments signal the approach of desperately needed relief.

Rapidly expanding knowledge of the neurobiology of chronic pain, including new insights into the differential roles of the ion channels within the primary sensory neuron electrogenisome in pain mechanisms (Fig. 3 and Table 2) and the first successful advances in gene therapy in preclinical models, mark the arrival of exciting translational opportunities. In the race for effective therapies, the identification, cloning and sequencing of ion channels and sensors of noxious signals and the characterization of their roles in neurobehavioural studies have been crucial milestones. These innovations go hand in hand with clinical data from individuals with familial chronic pain conditions that have uncovered the relevance of specific members of the electrogenisome as promising targets for traditional and emerging gene therapies[1,8,191]. Together with advances in functional genome studies, cell reprogramming and transcriptome regulation methods, the potential molecular approaches for genetic intervention have expanded in a variety of chronic pain conditions.

However, the swift pace of research has not yet yielded the desired clinical translation, which might be attributed, at least in part, to the



c

Peripheral sensitization	Reduction in threshold and/or increase in magnitude of responses of the peripheral endings of sensory fibres and/or cell bodies. For example, increased firing of nerve terminals in response to inflammatory mediators at a site of tissue injury
Ectopic neuronal activity	Abnormal spontaneous action potential activity in sensory fibres and/or cell bodies, in some cases due to effects of disease or injury on ion channels or presence of mutant channels
Gene dysregulation	Transcriptional changes or epigenetic alterations in sensory neurons causing dysregulation of channel expression or function, leading to sensitization of nociceptors
Presynaptic modulation	Pathologically enhanced neurotransmitter release from nociceptors due to augmented neurosecretory mechanisms or sprouting of sensory terminals
Switch of sensory modality	Acquisition of new modalities by sensory neurons after injury or drug treatment. For example, transmission of nociceptive signals by mechanoceptors (mechanical allodynia) after inflammation or high-dose chemotherapy

Fig. 3 | Neurobiological mechanisms underlying chronic pain in primary sensory neurons and related changes in their activity, presenting targets for gene therapy. a, Schematic illustration of the spinal cord with dorsal root ganglion (DRG) comprising primary sensory neurons. Through their afferents, DRG neurons conduct a variety of sensory signals from peripheral receptors to spinal neurons (SN) in the sensory areas of the spinal cord. **b**, Drawing of an enlarged DRG with three main types of primary sensory neurons: large proprioceptive neurons with fast conducting afferents (blue), medium-size mechanoreceptive sensory neurons with medium diameter afferents (magenta) and small pain-sensing sensory neurons with slow conducting thin afferents (red). **c**, Mechanisms of chronic pain in peripheral sensory neurons and underlying neurobiological processes leading to enhanced nociceptive signalling.

lack of objective pain readouts with reliable biomarkers, along with the marked placebo response in pain trials and complications related to biocompatibility and the limited precision of genetic interventions[9]. As a subjective experience, pain is influenced by many factors, such as psychological context, genetics, age and sex. Major efforts are currently under way to find specific biomarkers for various forms of chronic pain (peripheral and central components) that can engage different molecular cascades or cellular pathways depending on the source of pain (for example, tissue damage versus neuroplastic changes), associated metabolic dysfunctions (for example, diabetic neuropathy versus chemotherapy-induced neuropathy) and other factors[9]. New biomarkers based on electrophysiological and imaging readouts, analysis of biofluids or results of omic-based methods are expected to sharpen the focus on the most tractable molecular targets and aid in the evaluation of various therapeutic approaches in clinical trials[9].

Emerging advances in pharmacogenomics-guided 'precision medicine' approaches to pain could also provide an additional layer of specificity for gene therapy. For example, structural modelling, mutant cycle analysis and electrophysiological profiling of Na_V channels predicted the responsiveness of hyperexcitable DRG neurons to existing small-molecule blockers in vitro[192,193] and in studies assessing pain relief in human individuals in vivo[192,194]. Moreover, biophysical studies following-up from clinical trials on small-molecule Na_V blockers have begun to identify gene variants that confer a high level of pharmaco-responsiveness (versus a low level or non-responsiveness)[195], indicating the possibility of enhancing the overall efficacy of gene therapy approaches to pain by using hybrid and personalized medicine approaches in the future.

The advances described above have been paralleled by progress in tackling the immunogenicity and off-target actions of emerging gene therapies as well as by efforts to improve the precision of delivery and biostability of gene therapies to mitigate the risks of carcinogenesis[26,27]. The use of functionalized nanoparticles and carriers, synthetic receptors, and optimization of RNA-based therapies, among many others, are expected to address some of these challenges[196,197]. Future advances using these approaches not only are anticipated to facilitate the identification of appropriate gene therapy strategies and molecular targets, but also could personalize them for specific individuals to enable the most desirable outcome.

Given the expanding universe of therapeutic targets and the increasing number of gene therapy approaches, it is not unrealistic to suggest that there will be substantial progress towards more effective treatments for chronic pain in the foreseeable future. On the basis of the innovative research and technological improvements discussed in this Perspective, we believe that pain medicine is entering a new realm of more effective treatment that will be propelled, in part, by gene therapy.

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During the past twelve months, S.G.W. has served on the scientific advisory boards of OliPass Corp., Navega Therapeutics and Medtronic, and has served as an adviser to Sangamo Therapeutics, Exicure, Alnylam Pharmaceuticals, Chromocell, Ionis Pharmaceuticals and Replay Therapeutics.

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