Pharmacogenetics of new analgesics

Jörn Lötsch and Gerd Geisslinger

Pharmacogenetics of new analgesics (OPRM1, PTGS2) or associated signalling pathways (KCNJ6) could partly assign to the patients' genotype (Lötsch and Geisslinger, 2006; Lötsch et al., 2009a). Pharmacogenetic influences affecting the pharmacodynamic actions of marketed analgesics have been mainly found among the genes coding for their main targets (OPRM1, PTGS2) or components of their respective signalling pathways (KCNJ6), in a few additional genes (COMT, MC1R) partly affecting the function of the main analgesic targets, and in genes important for the pharmacokinetics of some classical analoges (CYP2D6, ABCB1) (Lötsch et al., 2009a).

With the targets of classical analoges, that is, opioid receptors and cyclooxygenases, and of substances labelled as co-analgesics, that is, the αδ subunit of voltage gated calcium...
channels, NMDA channels, sodium channels, noradrenaline or 5-HT transporters, there have been remarkable successes in treating pain. However, chronic pain has remained a primary healthcare problem listed by the World Health Organisation. Consistent with the multifactorial nature of pain (Julius and Basbaum, 2001), translational and genetic research have identified several new analgesic targets (Backonja and Woolf, 2010), for which analgesics are being developed (Table 1). From a pharmacogenetic point of view, this will increase the number of candidate genetic modulators of clinical analgesic actions, opening a chance of broader clinical use of genotyping information for pre-selection of analgesics.

The present overview summarizes potential pharmacogenetic modulators of those new analgesic targets for which substances have reached at least the clinical development phase 1. This avoids an inflated set of all possible targets that have not yet left basic research and their pharmacogenetic implementations are not yet acute. A complete set of all targets of analgesics that are presently considered as promising can, therefore, be found elsewhere (Marchand et al., 2009; Rodger, 2009). Due to corporate strategies and partially pending results, this survey does not provide a uniform picture of which compounds will be chosen for further development. Other new analgesics targeting the same structures as classic analgesics are excluded because this pharmacogenetic information has been discussed elsewhere (Lötsch et al., 2009a). Several novel analgesics were identified by a survey of online sources, including the http://www.clinicaltrials.gov database, company websites, presentations and press releases. As most of the new analgesics are nevertheless not yet broadly available, direct information about a pharmacogenetic modulation of their actions is often lacking. Nevertheless, pharmacogenetics can start from knowledge gathered in other context, often neuro-psychiatric disorders, where functional variants in the same genes have been already identified.

Pharmacogenetics of pain and analgesia in clinical practice

Pharmacogenetics are often expected to provide guidance for clinical drug therapy. This has been successful in several fields such as cancer therapy (Gonzalez-Angulo et al., 2010) or anticoagulation (Caraco et al., 2008), but pain therapy is not yet among them. The utility of genotyping information in clinical analgesia has been viewed from being broadly optimistic (Argoff, 2010) to pessimistic (Mogil, 2009); however, even most promising results on a modulation of common human analgesia are presently unable to provide a comprehensive prediction of individual analgesic response in the common setting (Lötsch et al., 2009c; Walter and Lötsch, 2009). Often, phenotypes could be only retrospectively associated with genotypes. This qualifies genotypes as risk factors and provides explanations for extreme phenotypes.

However, a prospective clinical utility has not been proven, as for example for CYP2C9 genotyping for warfarin anticoagulation (Caraco et al., 2008), neither are genotype-based analgesic therapy plans broadly used in clinical practice. Currently, most genotyping information has been associated with opioid requirements. Since opioids can be adequately efficiently titrated in most patients, there is no major advantage of genotyping information, beyond explanations for extreme dosing demands.

With the new, in-development, analgesics involving many new targets, the pharmacogenetics of pain and analgesia may be employed as guidance for the choice of the optimum analgesic. This is currently only marginally possible, as for example basing the non-selection of codeine on the CYP2D6 genotype (Eckhardt et al., 1998), the selection of a α-opioid agonist on the MC1R genotype and sex (Mogil et al., 2005) and the non-selection of a coxib on the PTGS2 genotype (Lee et al., 2006). Most of the new drugs are being developed against neuropathic pain and it is unlikely that a patient would receive all of these. Pharmacogenetic information may, therefore, be of great value in choosing the optimum analgesics, along with non-genetic, for example disease-specific, guidance.

Targets of new analgesics and their genetic modulation

Ion channels

Ion channels are integral membrane proteins that contain pathways through which ions can flow (Di Resta and Becchetti, 2010). They are considered likely targets in the treatment of pain (Mathie, 2010).

Voltage-gated sodium channels. Voltage-gated Na⁺ (NaV) channels are key mediators of neuronal function and essential for neuronal excitability (Mantegazza et al., 2010). They are the main targets of local anaesthetics. From a genetic perspective, the 1.7 subunit seems to play a major role in pain. Specifically, the complete inability to sense pain in otherwise healthy members of three consanguineous families from northern Pakistan was mapped as an autosomal recessive trait caused by a loss-of-function variant in the SCN9A gene (Cox et al., 2006). This gene encodes the α-subunit of the voltage-gated sodium channel, Na.1.7. In the three families, three distinct homozygous SCN9A nonsense mutations (S459X, I767X and W897X) were identified. In accompanying whole-cell voltage clamp experiments in HEK293 cells expressing mutant Na.1.7, voltage-gated Na⁺ currents were no greater than the background level. Additional very rare SCN9A variants have been added to the causes of this extreme phenotype (Nilsen et al., 2009).

The same gene also exhibits increased-function mutations, which cause the rare opposite phenotype erythromelalgia (Norbury et al., 2007; Choi et al., 2010) consisting of episodic symmetrical red congestion, vasodilatation and burning pain in the feet and lower legs. For example, a child with severe pain had a Na.1.7I234T mutation that induces a shift of –18 mV in the voltage-dependence of activation, accelerated time-to-peak, slower deactivation and enhanced responses to slow ramp depolarizations, with a –21 mV shift in the voltage-dependence of slow-inactivation (Ahn et al., 2010). Aside from these very rare genotypes, more frequent functional variants may modulate the pain phenotype of average carriers. The variant alleles rs6746030 A (Reimann et al., 2010) (frequency in Caucasians 9.7%) and rs41268673 T (Samuels et al., 2008) (frequency 1.4%) were reported as being associated with higher than average pain sensitivity.
Table 1
Compounds for which analgesia is the main clinical target, or at least among clinical indications, and which address a molecular target that has not been addressed by classical available analgesics or co-analgesics, or only as a pleiotropic effect, and which have reached at least clinical phase 1 in their development. The molecular targets and their coding genes are given as derived from publicly available information. Most substances are being developed for neuropathic pain.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Target</th>
<th>Gene</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Ralfinamide</td>
<td>Newron</td>
<td>Na,1.7, (and N-type calcium channels, NMDA)</td>
<td>SCN9A (CACNA1B, see below)</td>
<td>Missed primary endpoint (5/2010)</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>UCB</td>
<td>Na,1.8, 1.7 and 1.3</td>
<td>SCN10A, SCN9A, SCN3A</td>
<td>Suspended in 2007</td>
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<tr>
<td>MK-6721 / NMED160</td>
<td>Neuromed</td>
<td>N-type calcium channels</td>
<td>CACNA1B</td>
<td>Approved in 2004</td>
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<td>Ziconotide</td>
<td>Azur Pharma</td>
<td>KCNQ/Kv7 potassium channels</td>
<td>KCNQ2, KCNQ3</td>
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<tr>
<td>ACV1</td>
<td>Metabolic Pharmaceuticals</td>
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<td>Retigabine</td>
<td>Valeant</td>
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<tr>
<td>NGD8243 (MK-2294)</td>
<td>Ligand</td>
<td>TRPV1 channel</td>
<td>TRPV1</td>
<td>Partner suspended trial in 2008</td>
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<td>GRC-6211</td>
<td>Glenmark</td>
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<td>AMG986</td>
<td>Amgen</td>
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<tr>
<td>AMG8562 (back-up)</td>
<td>Amgen</td>
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<td>AZD1386</td>
<td>AstraZeneca</td>
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<td>NGX-4010</td>
<td>NeurogesX</td>
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<td>ABT 102</td>
<td>Abbott</td>
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<td>SB 705498</td>
<td>GlaxoSmithKline</td>
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<td>NGX-1998</td>
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<td>MK2295 (BGD8243)</td>
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<tr>
<td>NGX-4010</td>
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<td>adlea</td>
<td>Adesiva</td>
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<tr>
<td>GRC-15300</td>
<td>Glenmark</td>
<td>TRPV3 channel</td>
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<tr>
<td>Tezampanel (oral prodrug)</td>
<td>TorreyPines/Raptor</td>
<td>AMPA/kainate receptors</td>
<td>GRIA1-4/GRIK1-5</td>
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<tr>
<td>Indantadol (CHF-3381, V-3381)</td>
<td>Vernalis</td>
<td>NMDA receptor, MAO</td>
<td>GRIN1, GRIN2A-D, GRiNA, MAO</td>
<td>Suspended 5/2010</td>
</tr>
<tr>
<td>CNS-5161</td>
<td>Paion/ERGOMED</td>
<td>NMDA receptor</td>
<td>GRIN1, GRIN2A-D, GRiNA</td>
<td>Suspended 12/2009</td>
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<tr>
<td>RGH-896</td>
<td>ForestLabs</td>
<td>NR2B receptor subunit</td>
<td>GRIN2B</td>
<td>Phase 2 failed 6/2010</td>
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<tr>
<td>TC6499</td>
<td>Targacept/GSK</td>
<td>nAChR (α2/3β2) receptor</td>
<td>CHRNA4</td>
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<tr>
<td>ABT-594</td>
<td>Abbott</td>
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<td></td>
<td>Discontinued 2009</td>
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<tr>
<td>ABT-894</td>
<td>Abbott/NeuroSearch</td>
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<tr>
<td>EVT 401</td>
<td>Evotec</td>
<td>P2X7 receptor</td>
<td>P2RX7</td>
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<tr>
<td>CE-224</td>
<td>Pfizer</td>
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<tr>
<td>CE-535</td>
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<td>GSK</td>
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<tr>
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<td>Adolor/Pfizer</td>
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<tr>
<td>ADL5747</td>
<td>Adolor/Pfizer</td>
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<tr>
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<td>Pfizer</td>
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While these genetic changes have attracted the interest of pain researchers so far, the translation of the results of genetic research into drug development makes them immediate candidate variants for a pharmacogenetic modulation of Nav1.7 blocking analgesics. Carriers of increased-function variants might particularly benefit from Nav1.7 inhibitors because this would be a selective cure for paroxysmal pain (Fertleman et al., 2006). The consequences of carrying decreased-function variants are theoretically possible in both directions but could be easily assessed.

Voltage-gated calcium channels. Members of this ion channel family contain $\alpha$, $\beta$, and $\gamma$ subunits, and play a role in neuronal excitation. The $\alpha$ subunit of L-type calcium channels is the target of the established co-treatments for neuropathic pain, gabapentin and pregabalin (Perret and Luo, 2009). Newer developments such as ziconotide (Schmidtke et al., 2010), the synthetic form of the hydrophilic conopeptide $\omega$-MV2A found in the venom of the Pacific fish-hunting snail Conus magus (Olivera, 2006), target, with high affinity, the $\alpha_1B$ subunit of N-type voltage-sensitive calcium channels. These calcium channels are also key players in chronic pain (Swayne and Bourinet, 2008). They are coded by the CACNA1B gene and expressed at the presynaptic terminals of primary afferent neurons that end in the dorsal horn of the spinal cord (Gohl et al., 1994), an area playing a key role in nociceptive signal transmission. CACNA1B gave an above-threshold signal in a genome-wide association study of the risk of schizophrenia (Moskvina et al., 2009), and the gene was deleted in 16 cases of schizophrenia (Glessner et al., 2010).

Voltage-gated potassium channels. The inwardly rectifying potassium channel Kir3.2, a two-transmembrane-one-pathway potassium channel, is involved in opioid signalling on postsynaptic inhibition (Mitrovic et al., 2003) and mediates a significant component of analgesia (Marker et al., 2004). Delayed rectifying neuronal KCNQ channels (KCNQ2-5) have homologies with cardiac channels involved in long QT syndrome and play a role in benign idiopathic neonatal epilepsy or congenital deafness (Gribkoff, 2008). These slowly inactivating channels are also expressed at the postsynaptic membrane of small diameter nociceptive nerve endings (Brown and Passmore, 2009) and play a key role in the control of the excitability of nociceptors (Passmore et al., 2003). Potassium channels are considered as targets in several CNS diseases that involve neuronal hyperexcitability such as migraine, epilepsy or neuropathic pain (Wua and Dworetzky, 2005). KCNQ2/3 are not new analgesic targets as the well-known flupirtine has been recognized primarily to exert its analgesic actions via opening of these potassium channels.
Voltage-gated transient receptor potential (TRPV) channels. Members of this cation channel superfamily play critical roles in sensory physiology such as in vision, thermosensation, olfaction, hearing and touch (Montell, 2005). These receptors are activated by capsaicin (the pungent ingredient of hot peppers), protons and heat (>34°C), and are expressed at nociceptors and in pain relevant brain areas (Steenland et al., 2006). This stimuli are employed in experimental pain models using either directly heat and capsaicin (Petersen and Rowbotham, 1999) or producing protons via short pulses of gaseous CO₂ applied to the nasal mucosa, where protons are generated by the action of carbonic anhydrase (Kobal, 1985). A further family member, TRPV3, activated at temperatures of 22–40°C, is also expressed at sensory nerve endings (Ed and Cortright, 2009). Aside from these heat receptors, the TRP family includes cold receptors, among which TRPA1 and M8 have been most often associated with pain. TRPA1 is excited by cold stimuli below 15°C (Story et al., 2003), whereas TRPM8 channels are stimulated by cold between 8 and 28°C (McKemy et al., 2002). TRPM8 is also activated by cooling compounds such as menthol (Peier et al., 2002), while TRPA1 channels are additionally activated by pungent chemicals such as isothiocyanates (horseradish, mustard), cinnamaldehyde (cinnamon) and allicin (garlic) (Patapoutian et al., 2009) or cannabinoids (Jordt et al., 2004). New analgesics are either antagonists at the TRPV1 or TRPV3 nociceptors, or agonists (TRPV1) including capsaicin and chemically derived developments, which act via nociceptor desensitization (Novakova-Tousova et al., 2007).

Because of their involvement in pain sensations, TRPV1, A1 and M8 genotypes have been studied for modulation of the pain phenotype. Genetic associations have been reported about a single subject insensitive to capsaicin, who carried seven intronic TRPV1 polymorphisms and had only 50% of the mRNA and protein expression levels of normally sensing subjects (Park et al., 2007). In 17 European-American women carrying the TRPV1 variant rs8065080 G (I585V), cold withdrawal time was 1.6 times longer than in 136 non-carriers (Kim et al., 2004). This was surprising because TRPV channels are stimulated by heat and, therefore, heat pain was the phenotype expected to be modulated, while for a modulation of cold pain, variants in TRPM8 or TRPA1 would have been primary candidates. The authors hinted at properties in the three-dimensional structure of the engaged TRPA1 haplotype for explanation (Kim et al., 2004). In the same gene, a point mutation leading to an N855S amino acid exchange in the S4 transmembrane segment of TRPA1 receptors increased the inward current on activation at normal resting potentials fivefold (Kremer et al., 2010). This was associated with an autosomal-dominant familial episodic pain syndrome characterized by episodes of debilitating upper body pain, triggered by fasting and physical stress. For carriers of such TRPA1 mutations, TRPA1 antagonists are an especially promising therapy.
polymorphisms rs4603829 and rs4522666 were reported to modulate financial and psychological risk behaviours (Roe et al., 2009). CHRNA4 rs1044396 was associated with novelty seeking (Etter et al., 2009), whereas rs2236196, rs1044396 and rs2236196 were associated with nicotine dependence (Breitling et al., 2009). More severe consequences have some rare loss-of-function variants associated with the occurrence of amyotrophic lateral sclerosis (Sabetelli et al., 2009). However, as for several other candidate variants, a direct association with pain or analgesia has not yet been shown and possibly not even been studied because they have played, so far, no role in analgesia.

Purinergic receptors. Purinergic receptors have been reported to be involved in pain (Jarvis and Khakh, 2009; Jarvis, 2010). One mechanism by which ATP evokes acute pain is the interaction with P2X receptors that are also involved in the pathophysiology of chronic inflammatory and neuropathic pain (Kennedy, 2005). P2X3 receptors are expressed in capsaicin-sensitive small-sized dorsal root ganglion neurons where they contribute to the generation of rapidly desensitizing inward currents, which are involved in evoking nociceptive behaviours and thermal hyperalgesia. A P2X receptor-mediated excitatory postsynaptic current was also found in pyramidal neurones of the somatosensory cortex (North, 2003; Pankratov et al., 2003). P2X4 receptors induced in spinal microglia mediate tactile allodynia after nerve injury (Tsuda et al., 2003).

Genetic variation in purine receptors has been studied less frequently. P2RX7 rs1718119 was associated with severity scores in the panic- and agoraphobia scale (Erhardt et al., 2007) and the loss-of-function mutations P2RX7 568N (rs1653624), 307Q (rs28360457) and a null allele (splice site mutation, rs35933842) tended to be over-represented among patients who needed a surgical revision after total hip arthroplasty (Mrazek et al., 2010), which had been related to the involvement of P2X7 receptors in inflammation. In addition, a role in the development of depression has been suggested for the P2RX7 rs2230912 G allele (Nagy et al., 2008).

**G protein coupled receptors**

G-protein coupled receptors have seven transmembrane segments and sense external molecules triggering activation of internal signalling pathways.

**δ-opioid receptors.** δ-opioid receptors are the natural targets of enkephalins and mediate several biological functions including antinociception. Although the action of most current opioid analgesics is simply described as µ-opioid receptor agonism, it is well known that they also bind at other opioid receptors (Mignat et al., 1995). Therefore, a δ-opioid component is part of the action of most clinically used opioid analgesics (Gharagozolou et al., 2002). More selective δ-opioid agonists have not been broadly established clinically, despite demonstrations of better side effect profiles and a proposal of their use to selectively antagonize µ-opioid receptor associated respiratory depression (Su et al., 1998). Selective δ-opioid agonists are being under development as analgesics.

In *in vitro* transfection experiments, the W284L variant of the δ-opioid receptor selectively reduced the affinity of some but not all of the tested δ-opioid agonists (Hosohata et al., 2001). In vivo, most polymorphisms in the δ-opioid receptor gene (OPRD1) have been associated with substance dependence (Zhang et al., 2008) and other psychiatric disorders (Brown et al., 2007). For example, OPRD1 rs569356 may enhance transcription factor binding and increase δ-opioid receptor expression and was associated with substance addiction (Zhang et al., 2010). OPRD1 variants have also already shown to play a role in pain. Thus, men carrying the rs1042114T>G variant (allelic frequency 10.9%) had lower heat pain sensitivity than carriers of the rs2234918T>C variant (allelic frequency 35.6%) (Kim et al., 2004).

**Cannabinoid receptors.** The involvement of the cannabinoid system in a number of important physiological processes including the regulation of neurotransmitter release, pain and analgesia, energy homeostasis, and control of immune cell function is mediated by CB₁ and CB₂ receptors (Graham et al., 2009). They are activated by endocannabinoids, which are arachidonic acid derived lipids such as anandamide and 2-arachidonoyl-glycerol, plant cannabinoids such as tetrahydrocannabinol and synthetic cannabinoids including substances under development as analgesics. The two main receptor subtypes, CB₁ and CB₂, are primarily located in the CNS or in the periphery, respectively, although this separation is not strict, as, for example, the CB₁ receptor is expressed also in the lungs, liver and kidneys (Graham et al., 2009).

Cerebral endocannabinoid signalling is involved in antinociception (Wilson and Nicoll, 2002). However, the peripheral CB₂ receptor has also been proposed to play a key role in cannabinoid-mediated analgesia (Agarwal et al., 2007). Exogenous cannabinoids have been suggested to decrease the subjective intensity estimates of pain alone or in synergy with opioids (Naef et al., 2003; Roberts et al., 2006) but hyperalgesic cannabinoid actions on electric experimental pain stimuli in healthy volunteers have also been reported (Kraft et al., 2008). A cannabinoid formulation has been approved in Canada since 2005 for the treatment of neuropathic pain.

Polymorphisms in the CB₁ gene CNR1 have not yet been associated with pain phenotypes. So far, functional associations were found with obesity (Russo et al., 2007; Aberle et al., 2008), schizophrenia (Uijke et al., 2002; Chavarria-Siles et al., 2008; Hamdani et al., 2008), drug (Proudnikov et al., 2010) and alcohol dependence (Zhang et al., 2004; Zuo et al., 2007; Agrawal et al., 2009). Polymorphisms in the CB₂ gene CNR2 play a role in osteoporosis (Karsak et al., 2005) and might also modulate the susceptibility to autoimmune disorders (Sipe et al., 2005).

**Metabotropic glutamate receptors.** These Gₐ or G₂ protein coupled receptors transmit glutamatergic excitatory signals (Swanson et al., 2005), are expressed at nociceptive neurons and involved in sensitization processes to noxious stimulation (Coderre, 1993). Analgesics are being tested that inactivate the mGluR5 receptor, which is coupled with a G₂ protein. Via phospholipase C activation and inositoltriphosphate/diacylglycerol signalling, activation of this receptor leads to liberation of calcium from the endoplasmic reticulum into the intracellular space and to activation of
protein kinase C. GRM5 variants were associated with schizophrenia (Devon et al., 2001; Choi et al., 2009) or attention-deficit hyperactivity disorder (Elia et al., 2009).

Bradykinin receptors. Bradykinin B1 receptors (Marceau et al., 1998) mediate hyperalgesia due to kinin up-regulation (Gabri et al., 2006). In a diabetic neuropathic rodent model, blocking of B1 receptors reversed tactile and cold allodynia (Dias et al., 2007). A variant in BDKRB1 (-699 G>C) was slightly associated with progression of polycystic kidney disease (Tazón-Vega et al., 2007) but also with the risk of inflammatory bowel disease (Bachvarov et al., 1998). BDKRB1 variants were also reported in the context of hypertension (Cui et al., 2005).

5-HT receptors. 5-HT receptors are expressed in the central and peripheral nervous systems where they mediate both excitatory and inhibitory neurotransmission (Hoyer et al., 1994). They exert many physiological and pathophysiological functions and some of their subtypes play a role in nociception. Several 5HT receptor subtypes are involved in nociception (Xu et al., 1994), such as spinal 5-HT1, 5-HT3 and 5HT7 receptors (Alhaider et al., 1991; Danzebrink and Gehburt, 1991; Giordano, 1997). Human polymorphisms of their genes have been associated with several pathophysiological functions (Hannon and Hoyer, 2008) leading to a complex knowledge of the genetics and pharmacogenetics of the serotonergic system. Currently, only the 5-HT1A receptor is being studied as the target of an analgesic, F-13640/befiradol, that has entered the clinical phase of development. Besides several other biological functions in the regulation of blood pressure and penile erection, mood, addiction and memory, the 5-HT1A receptor subtype has been described to play a role in nociception (Nadeon and Goodchild, 2002; Pucadyil et al., 2005). An agonist at these receptors possessed anti-allodynic and anti-hyperalgesic properties (Bardin et al., 2003) including efficiency in neuropathic pain models in laboratory animals (Deseure et al., 2007). The HTR1A -1019C>G polymorphism was associated with schizophrenia, substance abuse disorder, panic attack and antidepressant response in mood disorders (Huang et al., 2004), attention deficit hyperactivity disorder (Shim et al., 2010), and has been suggested to be linked to frontal brain electrical asymmetry (Bismar et al., 2010).

Signalling messengers

Nerve growth factor (NGF). The nerve growth factor (NGF) is a small protein belonging to the class of neurotrophins and identified originally as a survival factor for sensory and sympathetic neurons in the developing nervous system (Fiore et al., 2009). The expression of NGF is high in injured and inflamed tissues, and activation of the NGF receptor tyrosine kinase A (trkA) on nociceptive neurons triggers and enhances pain signalling by multiple mechanisms (Hefti et al., 2006).

trkA is a catalytic receptor being approached as an analgesic’s target, (Wang et al., 2009), but the candidate compound has not yet entered phase 1 clinical trial. NGF signalling plays a role in the generation of pain and hyperalgesia (Levi-Montalini et al., 1996; Fiore et al., 2009) also because the local production of inflammatory cytokines up-regulates NGF (Hefti et al., 2006).

Due to the signal transduction pathway, the actions of NGF targeting drugs may be genetically modulated both at NGFβ level, the gene coding for NGF β, and at NTRK1 level, the gene coding for trkA receptors. Loss-of-function variants in the NGFr gene have been identified as the causes of extreme pain phenotypes consisting of complete congenital insensitivity to pain. Since NGF and its receptor trkA are involved in nervous system development and homeostasis, the genetic variants are associated with other neurological deficits. Thus, the hereditary sensory and autonomic neuropathy type V (HSAN-V) is characterized by a loss of pain perception, impaired temperature sensitivity, ulcers, and sometimes self-mutilation, with variable autonomic involvement (Hilz, 2002). All three affected members of a Swedish family were homozygous for a coding 661C>T SNP (R211W) in the NGF gene encoding NGF-β, which affects a highly conserved region of the protein (Einarsson et al., 2004).

Variants in the NTRK1 gene coding for the trkA receptor have been identified as the causes of the extreme pain phenotype congenital insensitivity to pain with anhidrosis (CIPA), also called hereditary sensory and autonomic neuropathy type IV (HSAN-IV). It is an autosomal-recessive disorder characterized by recurrent episodes of unexplained fever, anhidrosis, absence of reaction to noxious stimuli, self-mutilating behaviour and mental retardation. Since mice lacking the gene encoding the trkA receptor (ntrk1) for NGF display similar phenotypic features as CIPA patients (Smye et al., 1994), mutations in the human NTRK1 gene have been studied as candidate causes. Three mutations (1726delC with premature translational stop, IVS15 + 3A > C with altered splicing, 1795 G > C with G571R amino acid substitution) in three unrelated subjects were identified as the molecular basis of HSAN-IV (Indo et al., 1996). Several further mutations have been found in these patients (Indo, 2001), most of them only once but 1726delC was found in more than 50% of Japanese CIPA families (Miura et al., 2000), and 1926-1927insT found in 16 of 19 unrelated CIPA families from Israeli Bedouins (Shatzky et al., 2000).

Interleukin-1. Interleukin (IL)-1 is a pro-inflammatory cytokine. Antagonists are developed for the treatment of inflammatory rheumatic and low back pain. Genetic modulations in IL-1-related genes have been found in 131 middle-aged men, among whom carriers of the IL1 receptor antagonist (IL1R) variant rs2234677 had an increased risk for low back pain. When present with the IL1A genetic variant IL1A rs1800587 or the IL1-β gene (IL1B) variant rs1143634, a higher risk and more days with low back pain was identified (Solovieva et al., 2004). Higher pain incidence was associated with the IL1A rs1800587 and IL1R rs2234677 variants and the simultaneous presence of IL1A rs1800587 and IL1R rs2234677 was associated with increased number of days with pain (Solovieva et al., 2004). The functional variants were associated with IL-1 up-regulation at RNA and cytokine levels (Pociot et al., 1992; Dominici et al., 2002). This makes antagonists primary choices for patients carrying variants that enhance the algesic activity of IL-1.

P38 MAP kinase. P38 MAP kinases respond to stress stimuli, such as pro-inflammatory cytokines and cytokines and cellular stresses (Ashwell, 2006). P38 MAP kinases play a role in the
pathogenesis of neuropathic pain. In microglial signal transduction under chronic pain states, downstream effects of p38 produce inflammatory mediators (Ji and Suter, 2007). Selective p38 inhibitors are being clinically evaluated for the treatment of chronic inflammatory disorders including those involving pain (Cottrell et al., 2009). The MAPK14 gene coding for p38 (named MAP kinase 14) has so far not been positively reported from association studies.

Enzymes involved in the production of nociceptive or inflammatory mediators FAAH. The fatty acid amide hydrolase (FAAH) is one of the endocannabinoid metabolizing enzymes. It degrades the fatty acid amide family of endogenous signalling lipids including the endogenous cannabinoid anandamide (Bisogno et al., 2005), which among many other functions has been implicated in the suppression of pain. Lack of FAAH has been associated with a cannabinoid related hypoalgesic phenotype in mice (Lichtman et al., 2004). Moreover, FAAH has been implicated in the antinociceptive effects of paracetamol (Högestätt et al., 2005; Mallet et al., 2008) and other analgesics such as R-flurbiprofen (Ates et al., 2003; Bishay et al., 2010) affecting prostaglandin production and therefore, its polymorphisms may modulate the action of classical and new analgesics.

In pain, a tendency towards increased pain sensitivity associated with frequent FAAH alleles was seen in a cohort of 935 subjects. Cold pain intensity was up to 1.4-fold increased in men carrying the variant FAAH alleles rs932816 A, rs4141964 C and rs2295633 A, and carriers of the rs4141964 C allele had shorter (0.8-fold) cold withdrawal time than non-carriers (Kim et al., 2006). This would be compatible with increased enzyme activity leading to accelerated endocannabinoid degradation but the molecular consequences of these variants have not yet been assessed.

Future directions of the pharmacogenetics of analgesia

Pharmacogenetics of pain and analgesia as a research tool

The genetics of pain and analgesia has proven its value as a superior research tool to discover the role of molecular pathways in human nociception and analgesia. Quantitative sensory trait techniques in rodents (Abiola et al., 2003) have been successfully employed to identify molecular pathways of nociception leading to an increased understanding of pain and in some cases to the identification of new analgesic drug targets. Information about pain pathways from human research employed genotyping of patients with rare and extreme pain phenotypes, thus identifying indispensable components of the human nociceptive system (Oertel and Lötsch, 2008).

Variants in pain-associated genes can be employed to test whether a molecular pathway identified in laboratory animals is relevant in humans. Without modulator molecules that can be applied to humans, genetic variants functionally altering components of the pathway can be taken as a substitute. This requires, however, the demonstration of a molecular consequence of the genetic variant to avoid over-interpretation of an accidental positive association. A genetic association was used as a tool for a proof-of-concept in assessments where genetics was not in the focus. For example, the role of GTP cyclohydrolase (GCH1) activity in pain, first found in laboratory animals, was proven in humans using GCH1 genetic variants shown to decrease enzyme up-regulation and tetrahydrobiopterin production at the molecular level (Tegeder et al., 2006).

Genetic association studies can also be used as a tool to generate hypotheses but this requires molecular proof and replications of the findings. Genetics has also contributed to identify functionally relevant portions of the gene product, as for example for the μ-opioid receptor (Wolf et al., 1999). Importantly, a demonstrable molecular effect or at least a reproduction of positive associations in an independent cohort has become a scientific standard on which pharmacogenetics results may be based. This has not yet been shown for all so far known polymorphisms affecting pain or other clinical symptoms related to the present drug targets.

Further pharmacogenetically modulated analgesic targets

The present overview addressed those analgesics targets for which substances are closest to clinical use. It excluded targets for which no drug has so far reached clinical development, such as the GCH1 that may be used to delay the development of pain (Lötsch et al., 2009b). Similarly, T-type voltage gated calcium channels have been shown to play a key role in nociception (Bourinet et al., 2005; Zamponi et al., 2009). Furthermore, hyperpolarization-activated, cyclic nucleotide-modulated (HCN) ‘pacemaker’ channels play a role in the pathogenesis of neuropathic pain (Chaplan et al., 2003; Papp et al., 2006) rendering them possible further future targets of analgesics. Moreover, acid-sensing ion channels (ASICs) are activated by extracellular protons and can trigger acid-induced pain during inflammation or metabolic stress (Deval et al., 2010). They may be addressed with an existing experimental pain model employing administration of gaseous carbon dioxide to the nasal mucosa where via carbonic anhydrase, protons are generated and, along with TRPV1 activation, stinging pain is evoked (Kobal, 1985).

A further potential target is the inducible microsomal PG E2 synthase 1 (mPGES-1) that catalyses the formation of prostaglandin E2 (PGE2) from PGH2, a cyclooxygenase product from arachidonic acid. PGE2 represents an important pain mediator and its pain signalling effects are translated mostly via peripheral prostanooid EP, receptors and spinal EP2 receptors (Vanegas and Schaible, 2001), mPGES-1-deficient mice showed a reduced pain hypersensitivity and inflammation in some but not all models (Kamei et al., 2004). It therefore qualifies as a target of anti-inflammatory and analgesic drugs, although it is not clear for which diseases such treatment would provide a particular advantage (Rörsch et al., 2010). The PTGES2 gene polymorphism rs13283456 (R298H enzyme) has been found to reduce the risk of type 2 diabetes mellitus (Lindner et al., 2007; Nitz et al., 2007), perhaps through contribution of a lowered body mass index (Fischer et al., 2009).

This overview also excluded new analgesics that are innovative improvements of classic principles but do not add new
targets. For example, tapentadol is an opioid and noradrenaline re-uptake inhibitor, but its pharmacogenetics may be primarily deduced from known pharmacogenetic associations of OPRM1, KCNJ6 and COMT. Some further variants may be added in genes coding for targets of analgesics that have reached phase 2 without a publicly disclosed mechanism of action, such as the ‘small molecule’ AGN-209323. Candidate genes additional to the present variants affecting the pharmacodynamics of analgesics, may be found in drug metabolizing enzymes or transmembrane transporters potentially affecting the analgesic’s pharmacokinetics. However, from the present selection, those variants will be dropped for which the whole target fails clinical development. It is relevant to note that pain drugs under clinical development have failed, for example, ADX10059, RGH-896, raloxifene and tanezumab. Whether pharmacogenetic reasons played a role in these failures is not known.

Conclusions

For several genes coding for the targets of new analgesics (Table 1), functional modulations by genetic variants are already known. They have so far been found mainly in neuropsychiatric disorders and need to be tested for a possible role in analgesia. However, intensive research on the genetic modulation of pain has provided already substantial knowledge that may serve as a start point. That is, many targets have analysed in terms of pain genetics (Lötsch and Geisslinger, 2007), rather than analgesic genetics (Lötsch and Geisslinger, 2006), and may now be transferred from pain genetic research to pain pharmacogenetic research. Variants modulating the pharmacokinetics of new analgesics will possibly increase the number of candidates.

Several new analgesics will soon increase the choice of targets addressed for control of pain. This broader selection of analgesic targets and genetic modulators (Table 1) may increase the clinical utility of genotyping information in pain treatment, which so far with mainly opioid related proposed applications is modest (Lötsch and Geisslinger, 2010). The already considerable specific knowledge of functional variants, summarized here, may allow for specific hypothesis testing and help improving the statistical power of association studies that without a narrow selection of candidate variants would require large samples. Greater benefits of genotyping in pain therapy could be seen in the possibility to choose the individual optimum analgesic before the start of therapy. The chances for a genetics-based individualized pain therapy increase with an increasing number of targets. However, the challenge remains to compile this into clinically feasible guidance to therapy that provides additive value to therapy decisions made without genetics information.

Acknowledgement

Deutsche Forschungsgemeinschaft, KFO 129 to JL and GG, DFG Lo 612/10-1 to JL and DFG GE 695 to GG. We thank Dr Torsten Arndt for his help with the information gathering.

This paper was written using Free Software programs on an office and a home PC running Ubuntu GNU/Linux.

Conflicts of interest

The authors declare no conflict of interest.

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