Overview

Evolving Knowledge of Opioid Genetics in Cancer Pain

J. Droney*, J. Riley**, J.R. Ross***

* Imperial College, London, UK
** Royal Marsden Hospital, London, UK
*** Royal Brompton Hospital, London, UK

Received 12 November 2009; received in revised form 4 November 2010; accepted 22 April 2011

Abstract

Inter-individual variation in response to opioids for cancer pain is a well-established phenomenon. Variation occurs in the dose of opioid required, the analgesic efficacy of the opioid and also in the side-effects experienced by the individual taking the drug. To date, no clinical factor has been identified that can reliably explain or predict such variation. In recent years there has been growing interest in the possibility that genetic factors may play a role in the variability in opioid response. The aims of this review are to present the evidence supporting pharmacogenetic research in this area, to evaluate some of the studies and results that have been published to date and to present some of the challenges for future research in this area.

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Key words: Opioid; morphine; pharmacogenetics; single nucleotide polymorphism; variation

Statement of Search Strategies Used and Sources of Information


Introduction

In the past decade, pharmacogenetics, the study of the effect of an individual's genetic makeup on the response to medications, has yielded some very exciting results. An individual's response to drugs such as irinotecan, abacavir and warfarin can now be accurately predicted through integration of genetic and clinical data [1–5]. This facilitates prospective decision making regarding the choice of the correct dose of the correct drug for a given patient and reduces subsequent side-effects such as bleeding. In terms of cancer pain, pharmacogenetic studies have focused primarily on opioids. The aims of this review are:

- To present the reasons why some researchers and clinicians believe that the use of genetic information may, in the future, provide a valuable tool in improving cancer pain management;
- To review some of the cancer pain and opioid studies that have been published to date;
- To present some challenges that must be addressed if genetic data are to be meaningfully integrated in the clinical management of cancer pain.

This review focuses on the pharmacogenetics of opioids in cancer pain, with some discussion of relevant animal and non-cancer data when appropriate.

Inter-individual Variation in Response to Opioids

Morphine is the World Health Organization strong opioid of choice for moderate to severe cancer pain [6] and
for most patients morphine provides good analgesia without troublesome side-effects; these patients are ‘morphine responders’. There is, however, marked inter-individual variation in the overall clinical response to morphine [7]. A substantial proportion (up to 30%) of cancer patients are known as morphine non-responders [8,9]. These patients experience either inadequate analgesia despite escalating doses or intolerable dose-limiting side-effects [8,10,11]. Variability also occurs in the morphine dose required to achieve analgesia, both in cancer and non-cancer pain [12–14].

Pain perception and consequently analgesic response are complex clinical traits, potentially influenced by a number of different clinical and environmental factors, including age, gender, ethnicity, disease location, stage of disease, biochemical and haematological parameters, psychological status and concomitant medications. Researchers have attempted to identify clinical factors that are associated with morphine response/non-response. There have been few consistencies in the results of these studies.

In a retrospective study of cancer patients taking morphine, differences in patient age, white cell and platelet count were found when 100 ‘morphine responders’ were compared with 70 ‘morphine non-responders’. These factors only accounted for 6.9% of variability in the study population and that there were a number of limitations to the study due to its retrospective nature [15]. In another retrospective analysis, 103 cancer patients who underwent an opioid change (due to inadequate pain control, adverse effects from the opioid or other reasons) were compared with 170 patients who responded well to their initial opioid. In this study there was no difference in terms of age, gender, tumour diagnosis, pain syndrome or co-analgesics, with the exception of corticosteroids [16].

In a prospective study of cancer patients on morphine, regression analysis was used to develop a model to predict ‘morphine responders’ (n = 138) versus ‘morphine non-responders’ (n = 47). Morphine non-response was found to be associated with white cell count, weight, tumours of the lower gastrointestinal tract, recent chemotherapy and concomitant use of beta blockers, proton pump inhibitors or 5HT3 anti-emetics [8]. A further prospective study found no relationship between the need for an opioid switch (due to uncontrolled pain, adverse opioid effects or convenience) and age, gender, pain type, the use of adjuvant medications (including corticosteroids) or biochemical factors [17].

To date the only clinical factor that is consistently and reliably associated with altered morphine response is renal dysfunction. Morphine and its metabolites (including morphine-6-glucuronide) are eliminated via the kidneys [18,19]. Therefore, in renal impairment the effect of morphine is prolonged and more pronounced. However, marked inter-individual variability in morphine response occurs even in patients with normal renal function. Similar inter-individual variation has been described for other opioids, including fentanyl and methadone. At present, for the large majority of patients, no clinical factor has been identified that allows prospective prediction of which patient will respond best to which opioid.

Pharmacogenomics of Opioids

In recent years there has been a growing interest in the possibility that genetic factors might play a role in this variability of opioid response. There are significant animal data to support the hypothesis that pain and opioid response may be under genetic control. Morphine acts primarily through the mu-opioid receptor (MOR). MOR knockout mice (in which the MOR gene has been ‘turned off’) are entirely refractory to the analgesic effects of morphine [20–22] and do not experience any side-effects from morphine [21,23]. Other genetically bred strains of mice exhibit differential response to pain perception and opioid analgesia. For example, the CXBK mouse is a genetically inbred strain that has decreased MOR expression [24]. These animals experience markedly reduced analgesia from morphine compared with other mice [25]. Current research summarised in the Pain Genes Database suggests that there are nearly 300 genes, variation in which may play a role in differential pain perception and pain control in animals [26].

Human Opioid Pharmacogenetics in Cancer Pain

The best known example of the effect of genetic factors on opioid response in humans involves the metabolism of codeine. Codeine exerts its analgesic action primarily through its metabolism to morphine by the enzyme CYP2D6. A number of different variations in the gene coding for CYP2D6 have been identified [27]. These variations have been shown to be associated with altered morphine formation and the subsequent differential response to the drug. About 10% of Caucasians are known as ‘poor metabolisers’. These individuals have reduced CYP2D6 activity, make less morphine from codeine and thus experience reduced analgesia. On the other hand, ‘extensive metabolisers’ and ‘ultra-rapid metabolisers’ have increased CYP2D6 activity leading to enhanced morphine formation, with these patients being at risk of toxic side-effects [28–32]. Surprisingly, the effect of genetic variation in CYP2D6 on codeine response has not been extensively studied in cancer patients.

The opioid pharmacogenetic studies in cancer patients that have shown some positive results have primarily focused attention on three genes; OPRM, COMT and multidrug resistance 1 gene (MDR-1).

Genes are chosen for candidate gene association analysis because their end product (the protein they code for) is known to play a role in opioid response. All published studies of opioids and cancer pain involve examination of the effect of genetic variation at a DNA level. These studies explore any potential associations between single nucleotide polymorphisms (SNPs) and the response to morphine in cancer patients.

DNA is composed of two strands of nucleotides that run in the opposite direction to each other. SNPs are single point changes occurring at a known position along the structure of DNA. SNPs represent a change in the nitrogenous base making up that nucleotide; adenine (A), guanine (G),
and in human volunteer studies[36], although tri-allelic SNPs do also occur. SNPs may influence the clinical outcome through a change in the amino acid sequence produced by the gene, thus altering the structure or function of the enzyme/receptor/protein coded for by that gene. This is thought to occur in a number of ways:

1. The SNP may occur in a region of the gene (known as an exon) that is translated into protein.
2. Through a process known as linkage disequilibrium (non-random association), the SNP may represent a marker for the true disease-susceptibility SNP. In this way SNPs occurring in non-coding (intronic) regions of the gene may appear to be associated with changes in clinical outcomes in genetic association studies.
3. The SNP may occur in an area of the gene that subsequently alters the process and products of transcription (a necessary step in gene expression).

### OPRM

OPRM is the gene that codes for MOR. This is a natural candidate gene, as morphine and other opioids exert their effects primarily through this receptor.

Genetic variation in OPRM has been associated with variation in the opioid response in non-cancer studies, including postoperative pain [14,33,34], labour pain [35] and in human volunteer studies [36–38].

Over 800 polymorphisms in OPRM have been described [39]. The most commonly studied SNP is rs1799971. This SNP is also known as A118G, the two possible alleles at this location being A and G. The frequency of the polymorphism (the G allele) varies depending on the population being studied, from 16% in northern and western Europeans to 46% in Japanese [40]. A118G results in an amino acid change from asparagine to aspartic acid. This polymorphism occurs in the region of the gene that codes for the extracellular amino-terminus of MOR, the part of the receptor involved in ligand binding.

Lotsch et al. [41] studied the association of A118G with the central effects of morphine and M6G in a single-blind two-way crossover study of healthy volunteers (n = 12). Pupil constriction in response to intravenous administration of morphine or M6G was used as a measure of the central effects of the drug. Individuals carrying the G allele (GG or AG genotype) required larger doses of M6G to achieve 50% pupil constriction than subjects with the AA genotype. In this study there was no association between morphine response and A118G [41]. Another study of 20 healthy volunteers showed that higher doses of alfentanil were needed to achieve analgesia in subjects with the GG genotype than subjects with AA or AG genotypes [42]. In a larger cohort of the same study, carriage of the G allele (genotype AG or GG) at this position was also associated with increased morphine dose requirements (P = 0.012) [42].

In a study by Campa et al. [43], 145 cancer patients who were being started on morphine for pain were titrated according to their individual needs and were followed up prospectively for 8 weeks. Pain scores were assessed using an 11 point NRS and a verbal rating scale. At the end of week 1, patients with the AA genotype (n = 106) had a mean drop in pain scores from baseline of 3.73 ± 1.72 (mean ± standard deviation) compared with 0.3 ± 1.77 in those with the GG genotype (n = 10; P < 0.001) [43].

The clinical relevance of OPRM A118G is difficult to interpret at this point. There are a number of inconsistencies in the results of studies in this area. A single time point observational study by Ross et al. [11] showed no association between A118G (or any other OPRM SNP tested) and ‘morphine responders’ versus ‘non-responders’. In this study, morphine responders had been on morphine for at least a month and had good pain control without intolerable side-effects. Morphine non-responders had inadequate analgesia despite adequate morphine dose escalation and/or intolerable side-effects [11]. A study of fentanyl requirements in patients in labour suggested that the A allele (and not the G allele in other studies) is associated with higher fentanyl dose requirements (P = 0.0091) [35]. A recent meta-analysis of eight clinical studies (cancer and non-cancer) suggested that the association between OPRM A118G and opioid response does not actually withstand such an analysis [44]. In this meta-analysis, the study by Klepsstad et al. [12] was excluded in favour of the larger study by Reyes-Gibby et al. [42] because of the overlap in the study population. The Italian study was also excluded as the genotype frequencies were not in Hardy–Weinberg equilibrium, i.e. they deviated from expected frequencies.

A limited number of other SNPs across OPRM have also been studied in cancer patients on morphine. Klepsstad et al. [12] examined three other OPRM SNPs (−172 G>T, IVS2 + 31 G>A and IVS2 + 691 G>C), but these were not found to be associated with variation in the study end point, daily morphine dose requirements [12]. Ross et al. [11] examined six other OPRM SNPs in addition to A118G (−172 G>T, 5433 C>T, 32459 C>T, 50665 A>G, 51325 G>C and 80547 T>C). There was no difference in the frequencies of these SNPs between ‘morphine responders’ versus ‘non-responders’ [11].

There is also controversy about the functional relevance of the A118G polymorphism. One study showed altered receptor binding affinity for β-endorphin, but not for commonly used MOR agonists such as morphine or fentanyl [45]. However, another study reported no difference in
binding affinities for beta-endorphin between wild-type receptor and the MOR coded for by this variant, but did find binding differences for other MOR agonists [46]. A further study found no differences in binding of either morphine or beta-endorphin [47]. One study reported reduced mRNA and receptor expression associated with the G allele [48]. Therefore, overall, it is unclear whether A118G truly plays a significant role in inter-individual variation of the morphine response.

**COMT**

The COMT gene codes for catechol-O-methyltransferase, an enzyme involved in the metabolism of catecholamines, including adrenaline, noradrenaline and dopamine. These substances act as neurotransmitters in the brain. There is considerable evidence of interaction between the opioids and catecholamine systems [49–51]. Reduced COMT activity leads to elevated catecholamine levels, which have been associated with increased experimental pain sensitivity [50,52].

The most commonly studied polymorphism in this gene is rs4680, also known as Val158Met. This SNP is associated with an amino acid change from valine to methionine, which is associated with reduced enzyme activity [53]. One of the earliest genetic studies of this polymorphism genotyped 29 healthy volunteers and measured their pain response to saline infused into the masseter muscle. The volume of saline necessary to reach and maintain a certain level of pain intensity was used as a measurement of pain sensitivity. Patients with the met-met genotype had increased pain sensitivity, i.e. they experienced pain at lower volumes compared with those with the val-val genotype. Subjects homozygous for met also exhibited decreased regional brain opioid system activation [50].

Diatchenko’s group [54] subsequently identified COMT haplotypes (combinations of SNPs) that were associated with variability in pain sensitivity. It has been proposed that the COMT haplotypes alter mRNA structure [55]. Interestingly, the haplotype study did not find a significant association between pain sensitivity and Val158Met. Instead, the haplotypes associated with ‘low pain sensitivity’ and ‘high pain sensitivity’ both contained the allele at this position, which coded for the val variant, and it was the haplotype representing the intermediate pain sensitivity that had the allele coding for the met variant [54]. In a later paper the same group examined the influence of COMT haplotypes and the Val158Met SNP on different types of painful stimulus and concluded that although the haplotypes were associated with differential resting pain sensitivity, the Val158Met SNP was associated with the rate of temporal summation of heat pain [51]. Like OPRM, however, there are inconsistencies in the reported data. Kim et al. [56,57] did not find any strong association between Val158Met and experimental pain sensitivity (n = 500) or postoperative oral pain. In the latter study, the results of the COMT haplotype analysis also gave conflicting results to previous data.

Intriguingly, in two studies (n = 207, taken from the same study population) of the response to morphine for cancer pain, patients with the met-met genotype required lower doses of morphine than those with val-val (mean 24 h dose 95 mg met-met versus 155 mg val-val) [42,58]. As the met allele is associated with reduced COMT activity, it is associated with increased dopamine concentrations with subsequent suppression of enkephalins [49]. This is then associated with compensatory up-regulation of MOR expression [50,59]. The investigators therefore proposed that the opioid receptor up-regulation might be associated with an increased effect from morphine in met-met individuals with subsequent lower morphine dose requirements [58].

The same group also analysed haplotypes across the entire COMT gene and found an association between one haplotype (containing the A allele coding for met) and morphine dose requirements (P = 0.006). However, other haplotypes that also contained the A allele at this position were not associated with the variability in morphine dose [60].

The same study population was used to examine the joint effects of COMT Val158Met and OPRM A118G on variability in morphine dose requirements for cancer pain. Consistent with the group’s previous findings, they concluded that subjects with genotype OPRM A118G AA and COMT met-met required the lowest morphine dose (P = 0.012) [42].

One study in cancer pain compared inter-individual variation in response to oral morphine in terms of the development of central side-effects, showing an association between two intronic SNPS in COMT (rs7290221 and rs5746849), but not Val158Met, and an increase in confusion, drowsiness and hallucinations [61].

Overall, as for OPRM, the numbers of studies examining genetic variation in COMT in cancer pain are small and the data regarding genetic variation in COMT and opioid response are as yet incomplete.

**MDR-1**

MDR-1, also known as ATP-binding cassette B1 (ABCB1), codes for p-glycoprotein, which regulates the transport (efflux) of morphine from the brain into the blood across the blood–brain barrier [62]. Reduced p-glycoprotein levels/activity results in enhanced analgesia from systemically (but not centrally) administered morphine [63]. MDR-1 knockout mice exhibit an increased ratio of brain : plasma morphine concentrations compared with normal mice [64]. In animal studies, quinidine, a p-glycoprotein inhibitor, increases the concentration of morphine in the brain, with improved analgesic effect [65]. Similarly, pharmacological inhibition of p-glycoprotein enhanced morphine analgesia in rats with cisplatin-induced neuropathy [66].

Three MDR-1 SNPs have been commonly studied; C3435T (rs1045642), GT2677A (rs2032582) and C1236T (rs1128503). A study of 126 Korean patients treated with intravenous fentanyl found an association between MDR-1 SNPs C3435T and GT2677A and respiratory suppression (P = 0.0056) [67]. Subjects homozygous for the variant of SNP C3435T (genotype TT) have a reduced level of p-glycoprotein expression [68].

SNPs in MDR-1 have been examined in two studies of cancer patients taking morphine. C3435T was found to be
associated with variability in pain relief in an Italian study involving patients starting morphine for cancer pain. Patients with genotype TT (n = 38) at this position had greater pain relief than those with CC (n = 49; P < 0.001). In this study, the joint effects of OPRM A118G and MDR-1 C3435T were also examined. Patients with genotype OPRM A118G AA and MDR-1 C3435T TT seemed to have better pain relief compared with other combinations. However, the interaction analysis was not statistically significant [43]. GT2677A and C1236T, but not C3435T, were associated with a decreased level of central side-effects (drowsiness, confusion, hallucinations) in a study of 228 patients on morphine for cancer pain. In this study the protective alleles MDR-1 2677G and COMT 4873G were independently associated with a reduction in central side-effects on multivariate analysis [61]. There remains some controversy about the functional relevance of MDR-1 polymorphisms in opioid response [69–71].

Other Genes

The association between the response to morphine for cancer pain and a number of other genes has also been studied with mixed results.

One study examined the influence of cytokine gene polymorphisms on the analgesic response based on the idea that tumour-induced mediators such as cytokines may cause inflammation and modulate cancer pain. This study included 140 patients with lung cancer who were referred for pain management to the supportive care specialists. Subjects scored their pain on an 11-point NRS at baseline and 30 days after their first assessment. TNFα −308G>A was associated with variation in pain severity at 30 days (P = 0.04). IL-6 −174G>C was associated with differences in morphine equivalent daily dose (P = 0.004) [72].

An SNP in the gene coding for β-arrestin was found to be associated with differences between morphine responders and morphine non-responders (P = 0.013) [11]. β-arrestin is an intracellular protein that acts as an inhibitor of receptor coupling and is a negative regulator of opioid receptor signalling [73]. B-arrestin2 knockout mice have enhanced morphine algesia [74]. The same study also found that two SNPs in stat6, a gene involved in MOR transcription, were weakly associated with morphine responders versus non-responders.

An obvious candidate gene in pharmacogenetic studies of opioids is uridine diphosphate-glucuronosyltransferase 2B7 (UGT2B7). The enzyme coded for by this gene metabolises morphine to morphine-3- and morphine-6-glucuronide (M-3-G and M-6-G). A small study (n = 12) of patients using morphine via a patient-controlled analgesic pump found an association between an SNP in the promoter of UGT2B7 and morphine, M-3-G and M-6-G concentrations [75]. Polymorphisms in this gene were not found to be associated with concentrations of morphine or its metabolites in two larger studies in cancer patients (n = 70 and 239) [76,77]. Similarly, UGT2B7 SNPs were not found to be associated with any difference between morphine responders and non-responders [11].

There have been a number of other genes that have been analysed in animal, human volunteer or non-cancer pain studies that may play a role in opioid responsiveness, but have not to date been reported in cancer pain. These include melanocortin-1 receptor gene (MC1R) [78], GCH1 [79] and CYP2D6 [30].

Challenges in Carrying Out Genetic Association Studies in Cancer Pain

There are many challenges and issues in designing and interpreting any genetic association study. The pharmacogenetic studies of opioids in cancer pain highlight two of the main issues: (1) clinical phenotype definition and (2) sample size. Furthermore, these studies emphasise a particular challenge in pharmacogenetic studies in pain, that is, the clinical and genetic complexity of pain perception and analgesic response.

Clinical Phenotype Definition

In clinical practice there is no agreement as to how pain or symptoms should be assessed in cancer, with many units using a variety of different assessment tools [80,81]. This has a direct knock-on effect for research in this area. Although the number of studies in cancer patients taking morphine is low, there is wide variation and little consensus in the definition of a response to morphine, as detailed in Table 1. All the published studies examined the response to morphine alone, except for one study exploring the influence of polymorphisms in cytokine genes. In the latter study, patients were on a number of opioids, including morphine, oxycodone, fentanyl and methadone [72].

A number of studies examining the role of genetic variants on opioid response in cancer pain included objective measurements of morphine and the metabolites M-3-G and M-6-G [11,12,58,60,76,77]. However, no association was found between genetic variants and the metabolites. The heterogeneity in the classification of study outcomes in these studies makes it is difficult, if not impossible, to compare the data.

Pain perception and analgesic response are complex clinical traits [82]. In terms of the response to morphine, inter-individual variability in dose requirements, analgesic efficacy and the development of side-effects have been shown. Therefore, the response to morphine as a clinical phenotype or outcome represents a collection of non-independent phenotypes, all or any of which may be significant in any individual patient. Just as pain perception due to different pain stimuli may be under the control of different genetic variants [51], the genetic factors underlying each morphine response phenotype are probably quite different. This hypothesis is supported by the non-replication of the genetic findings across the different studies.

The situation is further complicated by the fact that, especially in cancer, there is a wide number of interacting factors (environmental, psychological, clinical and otherwise) that may confound or alter pain perception and the
response to analgesic agents. All of these confounding factors may be influenced by genetic factors. The design of case–control genetic association studies assumes that any noted change in genetic factors is directly related to the outcome measured [83]. However, this may not always be entirely true. For example, variation in a gene involved in mood may have an indirect effect on pain perception/analgesic response [84]. A number of the studies of opioids in cancer pain have tried to include or control for as many clinical and environmental confounders as possible. The Norwegian studies collected data on performance status, gender, age, tumour diagnosis, time on morphine, time to death, biochemical parameters, including serum albumin and creatinine, in the genetic analysis [12,42,60]. Ross et al. [11,61] also collected similar clinical data. There is a large number of other clinical and environmental factors that have been shown to influence pain perception or opioid response, including psychological state (anxiety and depression) [85,86], social status and diet [87]. Psychological state, namely depressed mood, was assessed in one genetic association study [72].

**Sample Size**

The power of any genetic association study is heavily dependent on sample size. Two of the main factors that determine the optimal sample size are (1) the size of the effect of the association between the genetic variant and the clinical phenotype (usually measured as the odds ratio or relative risk) and (2) the frequency of the genetic variant in the population being studied [88]. The smaller the effect size (i.e. the weaker the strength of association), and the lower the frequency of the minor allele, the larger the sample size required. Multi-factorial diseases/traits are influenced by multiple incomplete penetrant genetic variants, i.e. a number of individuals who carry the genetic...
variant of interest will not express the clinical phenotype [89]. Genetic variability is therefore probably associated with small or modest effect sizes (odds ratio or relative risk) in complex traits such as pain and analgesic response [90]. Studies that are too small run the risk of (1) being insufficiently powered to detect minor (but potentially important) associations between genetic variants and clinical outcome or (2) type I and type II errors [91].

The size of the study populations in the published genetic association studies of the response to morphine in cancer pain range from 70 [77] to 228 [61] (see Table 1). These studies were designed to test a limited number of SNPs in a limited number of candidate genes. The number of patients in these studies was therefore possibly adequate to allow the identification of possibly true associations in common alleles (allele frequencies >10% [89]) with more significant effect sizes. These study sizes probably precluded the identification of rare susceptibility alleles with smaller effect sizes.

The pharmacogenetic studies in cancer pain have all been hypothesis-driven candidate gene studies. Unless entire genes are resequenced in every individual (which is for the most part prohibitively expensive and laborious), candidate gene studies provide limited information because (1) only a small part of genetic variation (a limited number of SNPs) along a single gene is studied (as highlighted by studies involving OPRM above), (2) only a limited number of genes are examined and (3) if there are as yet unknown factors that are involved in the physiological processes of a disease or clinical trait, these will not be identified [90].

In recent years, many genome-wide association studies have been carried out, but none has been published to date in cancer pain. Depending on the platform used, these studies allow the simultaneous testing of up to one million SNPs in a hypothesis-free environment. One of the obvious challenges in carrying out such studies is that of recruiting an adequately sized study population to allow statistical correction for multiple testing. Without adequate corrections, there is a significant potential for false-positive findings [92].

**Pain and Analgesia are Complex Genetic Traits**

There are a few well-defined pain-related conditions in which a definitive causal gene has been identified. Mutations in SCN9A, which codes for a peripheral sodium channel, have been associated with either a complete inability to sense pain or, quite the opposite, an extreme pain disorder [93,94]. These are extremely rare conditions, however, and polymorphisms in the associated genes have not been identified as being associated with a variability in pain and analgesic response in general. Like other complex traits, pain perception and analgesic response are probably polygenic, resulting from multiple gene–gene and also gene–environment interactions [82]. In recent years is has been proposed that epistasis (gene–gene interaction) in disease pathogenesis and drug response is ubiquitous [95,96]. There may be a number of genetic variants that have a slight or even a negligible effect when analysed independently, but in the presence of another genetic or environmental variant, may be quite significant. Conversely, gene–gene interactions may in fact be antagonistic [96]. Gene–gene interactions are probably much more important and informative than single independent associations alone, especially in complex traits such as pain and analgesia. Association studies that focus on the association of single variants with pain and analgesic response probably produce ambiguous results and possibly miss clinically relevant genetic variants [97].

As detailed above, some studies have begun to examine SNP–SNP interactions in cancer patients taking morphine [42,43,61]. These studies have been limited to examining at most the interaction of two SNPs at a time. In each case these SNPs were chosen on the basis of previous candidate gene studies confirming a proposed clinical significance.

The concept of epistasis adds an enormous level of complexity, both practical and analytical, to the search for genes predicting response. A comprehensive search for all interactions would increase the required study sizes exponentially [98] and would probably only be possible through genome-wide association studies. The possible number of gene–gene interactions is as yet unknown and this carries the additional requirements for multiple testing corrections [99]. More sophisticated data analysis tools are required to deal with such interactive complexity. A number of statistical methods for examining gene–gene interactions have been proposed, including regression, classification and regression trees, neural networking, combinatorial partitioning and multifactor dimensionality reduction [96]. Even with the most robust statistical software, the sheer volume of data generated through combinations of polymorphisms is overwhelming. It has been suggested that the analysis of epistasis in genetic association studies should be guided by existing knowledge of plausible underlying biological pathways [100,101].

Although the number of pharmacogenetic studies of opioids in cancer pain has been low, the study populations have been quite distinct in terms of ethnicity and geographical location. This adds a further layer of complexity to any comparisons between studies, especially if the studies represent collaboration between different countries. Marked genetic variation between different geographical regions has been shown [102]. These differences may lead to spurious results in genetic association studies if the differences in allele frequency between cases and controls is due to population stratification rather than to the outcome being measured [83]. Such genetic heterogeneity may also be a reason why many genetic association studies fail to replicate in independent samples [92].

A further level of complexity is added by the fact that the effect of ever-changing clinical and environmental factors (e.g. disease progression, alterations in disease management, changes in psychological well-being) as well as the inevitable development of physical tolerance to opioids means that the relationship between the phenotype of pain perception and the response to opioids is dynamic rather
than static. The only prospective follow-up pharmacogenetic study in cancer pain was that carried out by Campa et al. [43], and even this study only involved data collected over a 1 week period in the genetic association analysis. All others involved assessment at a single time point. In terms of cancer pain, longitudinal follow-up studies and analysis are crucial to allow accurate prospective prediction of the morphine response.

Conclusions and Future Challenges

The current data supporting a role of genetic variation on response to opioids for cancer pain are limited. The studies have been small, examining only a handful of genetic variants and genes and the clinical phenotype definition has been too varied to allow definitive conclusions to be drawn. Furthermore, most of the studies have examined morphine alone, without considering the variation in response to other opioids. A recent study analysing the effect of the most commonly studied genetic variants on opioid response in a heterogeneous outpatient population concluded that, based on current knowledge, genotyping for current known genetic variants ‘barely merits the laboratory effort’ [103].

All of the pharmacogenetic studies of opioids in cancer pain have been limited to assessing genetic variation at a DNA level. Variation may also occur at an RNA level through alternative splicing, which is a process in which a single stretch of DNA can result in a number of different mRNA products, which may then result in variation in the protein produced. Alternative splicing is known to underpin the fact that multiple MOR subtypes exist [104,105]. These studies have been carried out in vitro, using cell lines. It has not been possible to carry out a clinical study in humans examining the direct association of variation in RNA with response to morphine for cancer pain because this would involve fresh brain and nervous tissue. A recent study by Diatchenko’s group analysed the effect of DNA SNPs on the mRNA and translational activity of OPRM isoforms. OPRM rs563649 was found to be located within an internal ribosomal binding site in an alternative exon of OPRM1 and the authors suggested that allelic variation at this site results in altered expression of a corresponding MOR isoform, MOR-1K and differential pain sensitivity. They also studied this SNP and haplotypes containing this SNP and the analgesic effect of morphine on experimental pain sensitivity, but the numbers included in this subset were small (n = 68) and the findings did not reach statistical significance, despite this SNP being implicated in the pain response in the same study in normal volunteers (n = 196) [106]. It is hoped that future studies might adopt a similar approach to further our understanding of the complex nature of genetics and the opioid response.

This review of pharmacogenetic studies of opioids in cancer pain has identified a number of challenges in carrying out genetic association studies, namely study size, phenotype definition and the analysis of complex traits. These challenges are not unique to studies of pain and neither are they insurmountable. Collaborations of investigators researching the same outcome would improve study power. Replication of results has traditionally been seen as the gold standard for validation. However, less than 20% of genetic associations have been robustly replicated without bias [107], often because of population stratification bias, population heterogeneity or inadequate statistical power [83,108]. These factors must be considered when designing any genetic association study. Exploration of underlying biological interactions and epistasis will enrich the search for genetic associations. Correlation of genetic data with functional studies will be a further step towards the validation of genetic findings. Identification and focused study of biologically plausible interactions between genes and the environment might yield more significant and useful results [98]. Hypothesis-free testing of hundreds of thousands of genetic polymorphisms brings us closer to finding the true ‘pain genes’, but do not negate the findings of other hypothesis-driven studies.

Further challenges include expanding the number of studies in this area with an emphasis on careful design, accurate clinical phenotype definition, adequate power and replication of findings. A comprehensive investigation of the genetic factors underlying inter-individual variation in response to opioids has the potential to revolutionise cancer pain management. Prospective prediction of the choice and dose of opioid would facilitate targeted and expedited pain management and promote rationale drug use for individualised patient care. Pharmacogenomics is a rapidly expanding field. The application of such advances in cancer pain is possible in the future, but not just yet.

References


