Genetics of neuropathic pain

William R. Lariviere
Department of Anesthesiology, University of Pittsburgh School of Medicine
3550 Terrace Street, Pittsburgh, PA 15261 USA

Abstract

Neuropathic pain affects numerous patients who have sustained damage to the nervous system. It remains unknown why only the minority of patients who sustain damage to the nervous system develops signs and symptoms of neuropathic pain, nor why treatments remain inadequate for many patients. This review highlights the evidence from animal studies of the genetic mechanisms of individual differences in the susceptibility to neuropathic pain-like symptoms and their removal. Microarray data, strain differences, genetic correlation analysis, linkage analysis, and pharmacogenetics are presented and
discussed. Putative genetic mechanisms have been discovered, instructive genetic relationships among neuropathic pain and other pain models have been detected, and important interactions between genetics and environmental factors determined. These findings provide us with directions and recommendations for further study of the genetics of individual variability in neuropathic pain and its treatment.

1. Introduction

Neuropathic pain affects numerous patients who have sustained damage to the nervous system. In some instances the damage is to neurons in the periphery, in others, damage is to peripheral neurons more centrally, near and in the spinal cord. Still others sustain damage to more central nervous structures due to trauma or cerebrovascular events. Neuropathic pain in humans is characterized by spontaneous pain, hypersensitivity to peripheral stimuli, and loss of function [1,2]. Examples include iatrogenic nerve injury, trigeminal neuralgia, post-herpetic neuralgia and neuralgia associated with HIV infection [3,4].

Although the precise etiological factors are not yet identified, several causes of neuropathic pain have been identified. Spontaneous activity and increased excitability of injured nerve fibers and neighboring fibers results in peripheral sensitization. In addition, ectopic excitation and phenotype switching of peripheral afferents, central sensitization, decreased inhibition, structural reorganization, and increased descending facilitation are all considered to contribute to the neuropathic pain syndrome [1,3,5]. Thus, it is generally agreed upon that multiple levels of change in the nervous system contribute and that multiple, heterogeneous mechanisms and combinations thereof are possible and likely in a given patient.

It remains unknown why many patients commonly sustain damage to the nervous system yet only a minority (up to 15%) develops signs and symptoms of neuropathic pain [6,7] accounting for several million patients in the United States alone [4]. Similarly, it remains unknown why only some patients respond optimally to currently available treatments, while the majority receives inadequate relief of their pain. Genetic mechanisms very likely account for a significant portion of the observed individual variability. This review will highlight the evidence from animal studies for individual differences in the susceptibility to neuropathic pain-like symptoms and their removal.

Animal models of neuropathic pain

Although it is recognized that significant differences exist between human and animal models of neuropathic pain, our current lack of understanding of the precise etiology leading to neuropathic pain in a subset of nerve-injured
patients warrants the continued extensive use of the latter. Animal models afford a much greater level of control over the nerve insult or injury, environmental influences, and genetic background of the subjects, facilitating the interpretation of mechanistic findings. Currently, this level of control is not possible in humans if we assume that patients have heterogeneous etiological factors and presentations of neuropathic pain.

Several animal models of nerve injury have been developed and are commonly used to examine the genetic mechanisms of neuropathic pain, including models of nerve transection, crush, ligation, or constriction, chemically induced nerve injury [8-12] and several variations thereof. The majority of animal models examine hypersensitivity to peripheral mechanical and thermal stimuli that is also seen in human neuropathic pain patients; the minority examines spontaneous pain behaviors. Notable differences among the animal models include variable involvement of immune responses, nerve regeneration, apoptotic mechanisms, variable degrees of denervation of the target tissue and associated trophic support, among other factors [13]. Although some investigators favor the use of particular models, there is some agreement that each model may facilitate determination of distinct and common genetic mechanisms that may be involved in human patients with multiple underlying causes.

Thus, the goals of genetics of neuropathic pain studies in humans and animals are to:

1) determine responses of genes after nerve injury;
2) determine genes related to individual differences in susceptibility;
3) determine genes related to individual differences in treatment effectiveness.

Achieving these goals should result in a more complete understanding of the fundamental mechanisms and facilitate individualized treatment of neuropathic pain.

2. Responses of genes after nerve injury

To obtain a fundamental understanding of neuropathic pain, the responses of genes following nerve injury have been studied in numerous publications. The vast majority of studies have examined single or a few genes that represent targets most likely to be involved based on pharmacological responses observed in human patients and animals, and on shift of emphasis in the field. Great emphasis has been placed on genes encoding ion channels, especially sodium and calcium channels [14]. Recently, emphasis has been placed on the role of glial cells in initiating and maintaining neuropathic pain [15,16].
In contrast to the method of selecting genes of interest for implication in neuropathic pain, current methods allow for the study of numerous genetic mechanisms with no *a priori* selection of the genes, including transcript microarray studies [17,18]. One advantage of microarray studies examining hundreds or thousands of genes is that in addition to identifying individual genes and their products as therapeutic targets, a more global understanding of the response to nerve injury can be obtained with the identification of classes of up- or down-regulated genes related to the multiple contributions to neuropathic pain. Commonly affected genes across species and neuropathic pain models can also be identified and may facilitate translation to humans.

**Microarray studies**

In possibly the most careful and detailed microarray pain study so far, Costigan et al. [17] simultaneously examined up- and down-regulation of 1000’s of genes in the rat dorsal root ganglion (DRG) after sciatic nerve transection using oligonucleotide microarrays for RNA expression. They identified a set of genes that are up-regulated in the DRG compared to expression levels in sham operated animals, including *GCH1* and *CCHA2* (*Cacna2d1*). In addition, they validated increased and decreased expression changes of 24 transcripts using additional methods of Northern blots, quantitative slot blots and *in situ* hybridization. The results confirmed previous reports of increased expression of *Cacna2d1* in the spinal cord and DRG after nerve injury, and further corroborate the efficacy of neuropathic pain medications gabapentin and pregabalin that target the gene’s protein product [19,20]. In addition, novel genes were also identified including *GCH1*, which might explain the efficacy of the antidepressant drugs currently used in the clinic that act on serotonergic and catecholaminergic neurotransmission since *GCH1* encodes for the rate-limiting enzyme for the synthesis of tetrahydrobiopterin (BH4), an essential cofactor for catecholamines, serotonin and nitric oxide production.

This study also establishes the technical details and standards by which subsequent and previous studies can be judged [17,18]. For instance, it was demonstrated quantitatively that the use of an overly conservative significance level or an overly conservative fold increase as the criterion for significant change markedly reduces the detection of transcript expression changes, especially for transcripts with low expression levels, or produces unacceptably high false positive error rates. They observed that combining an empirically determined acceptable level of significance and fold change maintained an estimated error rate of 5% and allowed for detection of 240 putatively regulated genes.

They were also able to group the up- or down-regulated genes into meaningful classes that correspond with our current understanding of the
response of the nerve to injury and the response of the nervous system that produces the pain phenotype. These classes were metabolism, regeneration, survival, excitability, neurotransmission function, sensitivity to the extrinsic and intrinsic signals; loss of trophic factors from peripheral tissues [17].

Partial overlap of these findings occurred with another microarray study of the DRG following sciatic nerve transection, which also detected significant expression changes in the transcripts for the peripheral benzodiazepine receptor (Bzrp), 5HT3, Gadd45, CLP36, Lysozyme, SNAP25, and VGF [21]. In a different neuropathic pain model of tight ligation of the fifth and sixth lumbar nerves, overlap with the Costigan et al. (2002) study also occurred with both detecting increased expression for Bzrp and Cacna2d1 [22]. A recent study provided the ideal comparison of mRNA transcript expression changes across three neuropathic pain models using the same microarray platforms and analysis methods [23]. The models were the lumbar spinal nerve ligation model (SNL), the spared nerve injury (SNI) model of transection of two branches of the sciatic nerve, and the chronic constriction injury (CCI) model of loose ligation of the sciatic nerve. In each of the models, 399, 184, and 310 transcripts, respectively, showed significant up- or down-regulation in the lumbar dorsal horn of the spinal cord. In common to all three models, were changes in 54 transcripts, most prominently several members of the complement cascade including C1q, C3 and C4 of microglial origin. The Valder et al. [22] study also detected significant changes in C1q and C4 expression. Thus, genetic mechanisms involved in multiple etiologies of neuropathic pain can be and have been identified.

Several limitations of microarray technology and methodology persist that necessitate validation of any results obtained. Array sensitivity limits require that significant differences be confirmed with PCR, immunohistochemical, in situ hybridization or other methods, especially for transcripts with low expression levels [17,18]. Although microarray studies have provided perhaps the best understanding of the fundamental genetic mechanisms of neuropathic pain, they do not directly assess the mechanisms of heritable individual differences in susceptibility to neuropathic pain that may be responsible for the marked clinical variability.

3. Individual differences in susceptibility

Strain differences: Heritability of neuropathic pain

Individual variability in susceptibility to neuropathic and other pain types is in part heritable [24-27]. Several studies have demonstrated the role of genetic background with significant effects of strain and substrain of rodent on susceptibility to develop spontaneous pain-like behaviors or hypersensitivity after nerve injury [22,25,28-32]. Even strains of rodents from different suppliers
that are in fact substrains with minor genomic differences show significant differences in neuropathic pain models [30,33]. Selective breeding of rats with high and low neuropathic pain behaviors has also shown that neuropathic pain is heritable and that the contributing genomic factors can be selected for [34,35].

In the autotomy model of neuropathic pain following sciatic nerve transection, nerve-injured animals exhibit self-mutilation of the denervated paws, biting at and amputating digits of the paw [10]. Significant strain differences in the percent of rats autotomizing within 7 weeks after nerve transection, the time of onset, and the maximal scores of self-mutilation have been reported among Sprague-Dawley, Brown Norway, Buffalo, Lewis and Wistar Furth strains [28]. The percent of rats of each strain autotomizing varied widely from 100% of Sprague-Dawley and Buffalo rats to 50% of Wistar-Furth to 0% of Lewis rats. The percentages varied roughly inversely with the mean onset of autotomy (2.0-3.5 weeks). However, both percentages and mean onset were not related to the mean maximal autotomy scores, suggesting that independent mechanisms underlie the divergent measures.

In the same study [28], immediate repair of the transected nerve by epineural suturing of the cut ends reduced the percent of animals autotomizing and the mean onset of autotomy behavior for all autotomizing strains. The relative rankings of each strain were maintained for the mean onset time and mean maximal score compared to autotomy without repair, potentially misleading one to conclude that the genetic background of the rats is of greatest importance across neuropathic pain models. However, the complete lack of autotomy behavior of Lewis rats in this nerve transection and repair study is not reflective of their susceptibility to other models of neuropathic pain including the lumbar nerve ligation model [30]. Tight ligation of the fifth and sixth lumbar nerves of Lewis rats produces noticeable hypersensitivity to mechanical stimulation with von Frey-type filaments and to cold stimulation with acetone application that is comparable to that observed for Sprague-Dawley rats [29,30]. In addition, Brown Norway rats that display higher levels of autotomy in the sciatic nerve transection model [28] show less spontaneous elevation of the nerve-injured paw and mechanical hypersensitivity in the nerve ligation model compared to Sprague-Dawley rats [30]. It is also important to note that even in the same neuropathic pain model and study, the relative ranking of strain sensitivity is dependent on the method of hypersensitivity assessment [25,30].

Mechanisms underlying strain differences

The mechanisms responsible for strain differences in neuropathic pain models are not yet known. Ongoing discharges originating in the neuroma of the complete sciatic nerve transection are variable between some rat strains
This could contribute to strain differences, although it has not yet been shown how closely related (and responsible) these discharges are for a wide range of strain differences in these models with both known peripheral and central contributions to neuropathic hypersensitivity.

It has been suggested that Lewis rats may not show autotomy in the sciatic nerve transection model due to their known decreased corticosterone levels and responses, especially compared to Fischer rats, since corticosterone levels are elevated in autotomizing rats compared to those that do not autotomize [28]. It may also be that their decreased autonomic nervous system (ANS) activity may contribute since manipulations of sympathetic nervous system function can modulate autotomy levels [38]. However, great caution is warranted in attributing strain differences to a specific known difference in one or a few inbred strains. Lewis rats also exhibit marked differences in behavioral responses to noxious and non-noxious stimuli and stressors that could equally affect pain behaviors [39]. For instance, Lewis rats predominantly freeze when placed in cold water instead of actively trying to escape as Fischer rats do [40].

Anatomical differences in the peripheral nervous system may also contribute to the strain differences in models of nerve ligation or transection. A recent, excellent report on species and strain differences in the anatomy of the sciatic nerve of the rodent has highlighted that more rostral lumbar nerves (L3 and L4) contribute the most to the sciatic nerve in the mouse compared to in the rat (L4 and L5) and provides clarification of probably mistaken nomenclature across many published reports [41]. The study also highlights that there are between-strain and within-strain differences in the relative contributions of the lumbar nerves to the sciatic nerve. Again, this factor of relative contribution to the sciatic nerve remains to be shown as responsible for reported differences across many strains. That is, it remains to be shown that the observed range of strain differences are physiologically relevant and explanatory considering the multiple levels of peripheral and central nervous system changes that contribute to neuropathic pain. However, the results do call for the careful quantification of the contribution of lumbar nerves in genetic studies of lumbar nerve injury.

Nonetheless, due to idiosyncratic strain differences (and environmental influences), we are severely restricted in interpreting differences among smaller inbred strain sets in pain traits as due to genomic differences or genetic mechanisms. Preferred methods are described just below.

4. Genetic relationships among pain types
Genetic correlation analysis
Larger inbred strain surveys of sensitivity to pain traits begin to directly address the specific genetic mechanisms underlying individual differences in
neuropathic pain models. The use of larger panels of fully inbred strains of rodents, also referred to as genetic reference populations, permits conclusions about genomic influences and genetic mechanisms. Well-characterized genetic reference populations, in which the genotype is fixed over time, experiments and across laboratories, permit direct comparisons across numerous studies of pain-related and non-pain-related traits [42,43]. These comparisons can be used to test the presence of an obvious confound such as strain differences in locomotor activity for behavioral pain studies, or baseline nociceptive sensitivity for studies of inflammatory and neuropathic hypersensitivity.

**Genetically distinct types of pain modeled in rodents**

A commonly used genetic reference population of mice are the standard inbred (SI) mouse strains. SI strains of mice have been used extensively to study the genetic mechanisms of individual variability in pain and analgesia traits [25-27]. As for outbred and inbred strains of rats, standard inbred strains of mice (e.g. C57BL/6, DBA/2, etc.) show striking differences in sensitivity in nerve transection or ligation neuropathic pain models that are consistent within strain [25]. Baseline nociceptive behaviors in response to mechanical, thermal, and inflammatory stimuli have also been examined in up to 12 of the same inbred mouse strains with heritability estimates in these assays ranging from 30 to 76% of the variability explained by the strain differences.

Genetic correlation analysis of these data has quantitatively determined the genetic relationships among sensitivity in numerous common assays, and have determined several genetically fundamental types of nociception and hypersensitivity [27,44]. In a genetic correlation analysis, the correlation between sensitivity of the inbred strains in the assays is calculated. Strong positive correlations indicate that common genetic mechanisms are responsible for sensitivity in the assays, whereas correlations close to zero indicate that distinct genetic mechanisms are responsible. Genetic correlation analysis has identified at least 5 genetically fundamental types of pain modeled: 1) baseline thermal nociception; 2) baseline inflammatory nociception; 3) mechanical hypersensitivity; 4) thermal hypersensitivity; 5) afferent-dependent hypersensitivity. The results also indicate that baseline mechanical nociception (in response to alligator clip application to the tail) is genetically distinct from mechanical sensation (in response to application of von Frey monofilaments to the plantar hind paw), and may be genetically distinct from baseline thermal and inflammatory nociception. Note that although three neuropathic pain assays were examined, they did not comprise their own clusters of assays; that is, they were not genetically distinct from all other, non-neuropathic pain models.
Relation of baseline sensitivity to neuropathic pain

With the hope of determining factors that predict susceptibility to neuropathic pain in humans, basal sensitivity to mechanical and thermal stimuli has been examined as a potential predictor. The relation of heritable susceptibility in neuropathic pain models to baseline nociception and hypersensitivity in other types of model can also be quantitatively determined from the results of standard inbred mouse strain surveys. This relation may provide some indication of the genetic predisposition of more sensitive individuals to particular types of neuropathic pain. The autotomy model of sciatic and saphenous nerve transection and the modified Chung model of tight lumbar nerve ligation have been examined in the above mentioned studies [25,27,44].

Thermal hypersensitivity in the ligation model was not correlated with baseline thermal nociception assessed with the same assay (Hargreaves’ thermal plantar test) nor with other thermal assays. In contrast, mechanical hypersensitivity (allodynia) in the ligation model is moderately correlated with baseline mechanical sensitivity assessed with the same assay (von Frey test) in the standard inbred strains, indicating at least partial common genetic mechanisms for the two. In a separate study, baseline mechanical sensitivity was not correlated with mechanical hypersensitivity following ligation of the sciatic nerve in a survey of eight inbred and outbred strains of rats [35], and may highlight an idiosyncratic relationship between baseline sensitivity and the etiology of neuropathic pain. Note that this analysis of predictability of baseline sensitivity provides an indication of predictability only in terms of shared genetic mechanisms; other non-genetic factors may contribute to each in variable ways.

Alternatively, the autotomy behavior in the nerve transection model is correlated with several thermal hypersensitivity models, all of which are correlated with baseline thermal nociception and all of which have been shown to be afferent-fiber dependent [27,44]. This is consistent with previous findings. The development of autotomy and the correlated thermal hypersensitivities have all been shown to be dependent on capsaicin-sensitive afferent fiber activity reaching the spinal cord and/or permanently inhibited by the local administration of anesthetic treatments [27]. This is in contrast to the lumbar nerve ligation model, which is only temporarily affected by local anesthetic administration.

Genetic relationships between neuropathic pain models

Microarray studies have found several common genetic mechanisms to be evoked by different neuropathic pain models [17,21-23]. However, heritable variability in autotomy behavior after nerve transaction is not genetically
correlated with mechanical or thermal hypersensitivity after lumbar nerve ligation across standard inbred strains of mice or after sciatic nerve ligation across strains of rats [27,35,44]. This indicates that distinct genetic mechanisms underlie individual variability in these two neuropathic pain models.

Paradoxically, mechanical and thermal hypersensitivity in the same lumbar nerve ligation model are not genetically correlated either, despite their induction by the same nerve injury [44]. A similar finding was reported in rats in which there was no correlation between mechanical and thermal hypersensitivity after ligation of the sciatic nerve [35]. This indicates that different measures of the consequences of the same nerve injury may have distinct genetic bases, as suggested by the simpler rat studies discussed earlier.

The mechanical and thermal hypersensitivities after nerve ligation are, however, correlated with intrathecal dynorphin-induced mechanical allodynia and subcutaneous carrageenan injection-induced thermal hyperalgesia [27]. Thus, genetically correlated non-nerve injury models may provide additional information regarding shared genetic mechanisms related to neuropathic pain.

In summary, rodent strain surveys of the neuropathic pain models examined so far indicate that distinct genetic mechanisms underlie heritable individual variability in neuropathic pain of different etiologies. In addition, different symptoms of neuropathic pain of the same origin are also likely mediated by distinct genetic mechanisms. Thus, heterogeneous patient populations may have a variety of predisposing genetic mechanisms, depending on the etiology of the neuropathic pain and the chief symptoms.

5. Linkage analysis: Quantitative trait locus (QTL) mapping

Towards identifying the precise genetic mechanisms underlying individual variability in neuropathic pain, QTL mapping has been used to determine regions of the genome associated with autotomy following sciatic and saphenous nerve transection [45]. Using a survey of 23 AXB/BXA recombinant inbred (RI) mouse strains derived from a reciprocal cross between A/J (A) and C57BL/6J (B), the frequency of male mice significantly autotomizing within each RI strain was compared to the status of 395 polymorphic DNA markers across the genome. In this linkage analysis, a DNA marker (D15Mit28) on chromosome 15 at approximately 43.7 cM (~74.95 Mb) from the telomere exhibited the greatest association to the autotomy trait, indicating that a linked gene or genes in the region is associated with autotomy. This QTL was named pain1.

The marker-autotomy association above did not reach the most stringent level of statistical significance for genome-wide mapping [46-48], but was
Genetics of neuropathic pain

statistically suggestive. The AXB/BXA RI strain means from the above-mentioned study have been placed in a public, online archive (GeneNetwork: www.genenetwork.org) [49] permitting the re-analysis of the QTL mapping results with a current, updated DNA marker set containing 2446 polymorphic markers useful for QTL mapping in these strains. Re-mapping the frequency of autotomizing mice within each strain with 1000 permutations showed a peak at the same relative position on chromosome 15 (approximately 74.5-75.5 Mb), but with a maximum LOD score below statistically suggestive levels for this genome-wide scan (suggestive LOD = 2.27).

Without confirmation, a suggestive QTL would not be considered worthy of follow-up. However, the pain1 QTL has been confirmed with a combined set of male and female mice from a backcross of C3H/HeN and C58/J strains [46]. The marker with the highest LOD score (3.0) in this study (of 9 markers along chromosome 15), and thus the highest association with autotomy behavior (D15Mit68) is located at approximately 44.1 cM (~76.7 Mb). Even though the two studies used slightly different mapping strategies and different inbred mouse strains, the same relative chromosomal region was detected in both the Devor et al. [46] and Seltzer et al. [45] studies, lending credibility to the reported QTL and justifying an intensive search for the responsible gene or genes.

The candidate gene or genes responsible for pain1 have yet to be identified. Candidate genes proposed in the original Seltzer et al. study based on known roles in nerve injury or related phenotypes were: Pvalb, encoding for parvalbumin; Bzrp (Tsopo), for the peripheral benzodiazepine receptor; Emo2, for emotionality traits in mice; Galr3, for a galanin receptor; Il2rb, for the beta chain of interleukin 2; Cacng2, for the gamma 2 subunit of a voltage-dependent calcium channel; Kcnj4, for the potassium inwardly-rectifying channel; Jrk, for a centromere binding protein. Unfortunately, the non-significant results from re-analysis on the GeneNetwork website and the interval at 40-48 cM on chromosome 15 suggested by the Devor et al. [46] study do not allow for restriction of the list of candidate genes and may suggest expansion of the list. Indeed, a later study found a marker at 32 cM on chromosome 15 to be associated with autotomy in female mice (LOD score = 3.3) [31]. However, two microarray studies have reported the up-regulation of Bzrp [17,21] making this gene an excellent candidate for the individual differences.

A recent attempt to find the homologous QTL of pain1 for autotomy in rats found a statistically suggestive QTL on chromosome 2 of the rat (named pain2), but failed to detect any QTL in regions of chromosomes 2 and 7 homologous to mouse chromosome 15 [50]. The pain2 QTL is in a region of chromosome 2 of the rat homologous to a region on chromosome 13 of the mouse. This study used 42 male and female mice of an F2 cross of lines of rats selectively bred for high autotomy (HA) and low autotomy (LA) behaviors and
a smattering of markers polymorphic among the F2 mice (7 each on chromosomes 2 and 7). Although the authors state that the pain1 QTL (and the linked genes) may still exist in the rat, no evidence exists yet for this conclusion. Nonetheless, a different set of candidate genes are proposed to underlie pain2 at present [50], although confirmation of the suggestive pain2 QTL is recommended prior to extensive candidate gene testing.

**Interactions of genetics with environment**

Perhaps hindering the quick identification of the genes underlying heritable variability in rodent neuropathic pain models and underlying pain1, are the effects of environmental factors and interactions between genetic and environmental factors. Although the use of inbred strains with fixed genotype and homozygous alleles in numerous studies is expected to significantly reduce variability in nerve injury models, a wide range of susceptibility within strains is commonly reported [28,31], and is perhaps responsible for the common neglect of variance measures in many of the published articles. Mice of the same strain in the same experiment often exhibit autotomy scores at the extremes, even exhibiting a bimodal response pattern [45,46]. This suggests that environmental factors continue to have a strong impact even when the genotype is controlled and fixed.

Housing conditions and the genotype of cage mates has been shown to influence susceptibility in neuropathic pain models [51]. In another genome-wide QTL mapping study of autotomy following sciatic and saphenous nerve transection using 370 mice of a backcross of C3H/HeN and C58/J strains, Devor et al. [31] were unable to replicate the pain1 QTL in male (n = 22) or female (n = 118) mice group-housed with 2-6 other mice of mixed genotype resulting from the backcross. Insufficient power may explain the results in the small sample of male group-housed mice. However, housing mice or rats of one inbred genotype that displays low autotomy with conspecifics of another inbred genotype that displays high autotomy can significantly increase autotomizing of the former group (and slightly decrease autotomizing of the latter) [31,51]. Thus, for the group-housed female mice, the presence of mice with different genotypes and different levels of autotomizing may have introduced sufficient environmental variability to prevent detection of a significant marker-phenotype association, whereas in isolated females a significant QTL was detected for a marker at 32 cM on chromosome 15 in the general region of pain1. Although the study showed that males of standard inbred strains show greater variability in autotomy than females, it is not easy to explain this study’s inability to replicate pain1 in males since the original detection of pain1 was with the use of only male mice (also displaying significant within-strain variability), albeit of a different genetic background (AXB/BXA RIs)[45].
These findings call for the careful design of experiments to minimize the impact of environmental (diet; caging) and other confounding variables including sex. Environmental variables including diet (protein type and concentration) can significantly increase or decrease the development of neuropathic pain behaviors in rodents [52,53], and interactions of diet with genotype have also been shown [54]. Possibly of even greater relevance to humans is the impact of prior sensory and pain experience. Prior injury and previous neurectomy experience has been shown to affect neuropathic pain development [55,56]. In addition, prior anesthetic block reduces autotomy behaviors [57].

Therefore, in human studies it will be critically important to control for prior history of injury and familial history of chronic pain including neuropathic pain. Because diets vary widely in people, and it is difficult to control for this factor, human studies should strongly consider measuring dietary content of at least protein types and concentrations as a predisposing or contributing factor.

6. Individual differences in therapeutic responsiveness

A number of drugs are used in the treatment of neuropathic pain in patients [4]. These include non-steroidal anti-inflammatory drugs, opiates, lidocaine patches, the antidepressant drugs duloxetine and venlafaxine, and the anticonvulsant drugs carbamazapine, lamotrigine, gabapentin and pregabalin. Some of the treatment effects have been related to changes in genetic mechanisms (for e.g., \textit{Cacna2d1}, \textit{GCH1}), as mentioned earlier. Successful treatment, however, remains inadequate for the majority of patients [3]. Genetic mechanisms likely contribute to this situation.

Just as many patients show sympathetically maintained neuropathic pain and are helped by treatments aimed at the ANS, animal models of neuropathic pain also show ANS dependence [58]. ANS modulating drugs and treatments including sympathectomy by guanethedine treatment and the monoamine oxidase inhibitor pargyline can decrease or increase signs of neuropathic pain in rodents, respectively [59,60].

However, treatments are not equally effective across strains of rodents with different genetic backgrounds. For instance, phentolamine, an alpha-adrenergic receptor antagonist is most effective in reducing evoked mechanical hypersensitivity in the L5/L6 lumbar nerve ligation model in Lewis rats and not at all in Fischer rats [29]. Although not yet definitively determined, such strain differences could be due to strain differences in projections of noradrenergic coeruleospinal neurons [61,62].

It is not surprising that heritable factors modulate the effectiveness of treatments on neuropathic pain. An effect of strain of mouse is observed for numerous analgesic drugs in a variety of pain tests [63,64]. The neuropathic
pain drugs gabapentin and pregabalin are no exception [65,66]. What may be most clinically relevant is that the pharmacogenetics of analgesics is also dependent on the type of pain. Standard inbred strains of mice show marked differences in the ability of gabapentin and pregabalin to counter inflammatory nociception in the formalin test and inflammatory hypersensitivity in the zymosan test [65]. While the two drugs show positively correlated analgesic sensitivity across the inbred strains when tested in the same pain test, the effectiveness of the same drug (pregabalin) is not correlated across the two pain tests [65], highlighting the importance of the type of pain being countered on the pharmacogenetics of the drug. Further support for this conclusion comes from studies that show the effectiveness of drugs of several distinct drug classes with distinct molecular targets to be positively correlated across inbred strains of mice when tested in the same thermal pain test (tail withdrawal) [64]. These results suggest that a systems genetics approach is needed to complement receptor-oriented pharmacogenetic studies.

Comparisons of analgesic effectiveness across neuropathic pain models demonstrates the specificity of effects for type of neuropathic pain and symptoms. In the two neuropathic pain models of sciatic nerve crush and transection of two distal branches of the sciatic nerve, gabapentin effectively prevents the development of tactile hypersensitivity in Sprague-Dawley rats in the former model but is not effective in the latter model at the same and higher doses [67]. Similar differential effects of drugs of other classes including morphine, amitriptyline, carbamazepine, and MK801 (an NMDA receptor antagonist) were also observed between the models [67]. Others have reported effectiveness of gabapentin in other neuropathic pain models following intracranial administration of gabapentin emphasizing the role of more central nervous processes in addition to those in the periphery and spinal cord [68]. Still others have demonstrated with ANS-directed treatments that the individual signs of neuropathic pain show differential sensitivity to modulation by the same treatment. Heat and cold, but not mechanical, hypersensitivity show sympathetic dependency in the chronic sciatic nerve constriction model (CCI) [69,70], whereas mechanical hypersensitivity in the partial sciatic injury (PSI) model of transection of two distal branches is reduced by ANS-directed treatment [71].

Thus, there are still unknown mechanisms of interactions between heritable factors of susceptibility to a particular type of nerve injury, heritable factors of individual symptoms of the nerve injury, and heritable factors of analgesic effectiveness that are dependent on the type of pain being modified.

7. Conclusions and recommendations

Studies in animals clearly indicate that susceptibility to neuropathic pain is heritable and that gene-by-environment interactions exist. With QTL
mapping, regions of the mouse genome associated with neuropathic pain behaviors have been identified but the responsible genetic mechanisms remain to be identified. Numerous genes that respond with up- or down-regulation have been identified for specific neuropathic pain models. However, the precise genetic mechanisms of individual variability remain to be identified. There are still unknown interactions between heritable factors of susceptibility to a particular type of nerve injury, of individual symptoms of the same nerve injury, of analgesic efficacy and effectiveness, and their dependence on the type of pain being modified. These heritable factors and interactions may underlie the clinical observations of inadequate treatment for many patients; the lack of generalizability of drug effectiveness across neuropathic pain types; and that drug class is not predictive of efficacy in clinical populations.

Although the direct translation of findings from animal studies to specific patient populations is not possible at present, the use of animal studies remains critical. Significantly greater control of environmental factors, genetic background, and etiology of nerve injury is possible in animal models. Even with this level of control, the evidence shows that the possible interactions among these factors is very complex and still requires extensive study. In human populations, little control of any of these factors is possible, which calls for massive cohorts to be able to parse out the effect of each of the variables in a genome-wide association study (GWAS), for instance. At present, evidence obtained from a well-designed human study may be difficult to interpret. This calls for the need to perform parallel human and animal studies to facilitate translation of findings to humans, including by prioritizing short lists of candidate genes from animal studies and, thereby, permitting studies with smaller human cohorts. Multiple end-points will have to be similarly studied in both humans and animals. So far, similar relationships have been observed across mouse and rat species. The same relationships may also hold true for humans.

Identification of genetic mechanisms in common to neuropathic pain of various etiologies is critical for the fundamental understanding of neuropathic pain. However, since neuropathic pain models and symptoms are in many cases genetically distinct, it may not be the correct approach for the development of the optimal treatment for a particular etiology and symptom of neuropathic pain. Indeed, generalizability of drug effectiveness in patients is not seen across neuropathic pain types [3,4]. Thus, it should also be a priority to identify the genetic mechanisms of heritability of neuropathic pain in each of the available neuropathic pain models that are genetically distinct. This would ensure that the distinct mechanisms can also be targeted in patients with presumably heterogeneous etiologies and symptomatology.
Identification of baseline sensitivity measures as predictors of neuropathic pain might also not be as fruitful as previously thought. Animal studies suggest that only the minority of neuropathic pain models and only a subset of the symptoms are correlated with baseline sensitivity. On the other hand, if only a few of these relationships exist, they may facilitate mechanistic identification of neuropathic pain type in humans, which may in turn instruct on the optimal treatment modality.

A systems genetics approach can be used to link the study of the genetics of mechanisms of subtypes of neuropathic pain, their multiple symptoms, and pharmacogenetics. Genetic reference populations such as standard inbred (SI) and recombinant inbred (RI) strains of mice represent an ideal model for this purpose. Genetic correlation analysis of SI strains (and inbred and outbred rats) has already provided the richest information regarding the genetic distinctiveness of neuropathic pain models compared to others, baseline nociception, and other hypersensitivity models. This line of research needs to be complemented by an intensive study of neuropathic pain models that have yet to be surveyed in SI mice, of effectiveness of analgesic drugs commonly used for neuropathic pain and of new drugs targeting novel genetic mechanisms identified in expression studies. The SI mouse model can also be used to identify regions of the genome associated with each of the traits using haplotype mapping and newly available single nucleotide polymorphism (SNP) databases for these strains [72], although there are limitations of power to detect associations with about a dozen or two of SI strains [73].

The RI mouse model can provide greater power to detect associations of genomic polymorphisms with neuropathic pain traits, and is currently used to study the systems genetics and ‘genetical genomics’ of other traits [74]. RI mice have been used for QTL mapping of neuropathic pain (for the detection of pain1) and other pain traits [45,75,76]. This genetic population, and particularly the BXD RI strains, has a rich archive of polymorphic markers across the genome and newly developed strains providing unprecedented genomic resolution for QTL mapping [77]. In addition, over 800 phenotypes already examined in these strains are available for genetic correlation analysis, and mRNA microarray data for several brain areas are available for transcript covariance analysis of pain traits with tissue-specific expression profiles [78]. As a genetic reference population, the relationships among neuropathic pain traits, genomic variability, and expression profiles can be performed quantitatively with this single model. This is currently not possible with human subjects and will not likely be possible for many decades. Recent efforts are capitalizing on this model to obtain convergent evidence from QTL mapping and transcript covariance analysis for specific brain areas to identify genetic mechanisms of several pain traits, including of mechanical sensation that may be shared with those of a specific neuropathic pain model [79-83].
Genetic mechanisms of variability in neuropathic pain remain to be determined, but animal models have provided valuable information regarding the genetic relationships among types of pain and symptoms, pharmacogenetics, the relation to baseline sensitivity, the role of environmental factors, and important experimental design issues. Concerted effort to translate these and future findings to humans may provide translatable information in the near future.

Acknowledgements
This work was supported by grants from NIDA (RO1 DA021198) and the University of Pittsburgh Schools of the Health Sciences Bridge Funding.

References
51. Raber, P., and Devor, M. 2002, Pain, 97, 139.