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Genetic architecture of human pain perception

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Pain is emotionally detrimental and consciously avoided; however, it is absolutely crucial for our survival. Pain perception is one of the most complicated measurable traits because it is an aggregate of several phenotypes associated with peripheral and central nervous system dynamics, stress responsiveness and inflammatory state. As a complex trait, it is expected to have a polygenic nature shaped by environmental pressures. Here we discuss what is known about these contributing genetic variants, including recent discoveries that show a crucial role of voltage-gated sodium channel Nav1.7 in pain perception and how we can advance our understanding of the pain genetic network. We propose how both rare deleterious genetic variants and common genetic polymorphisms are mediators of human pain perception and clinical pain phenotypes.

What is pain?

Pain is an unpleasant and emotionally arousing sensory experience that invades our consciousness. The perception of acute pain is an adaptive response that signals the presence of damaging and life-threatening events (e.g. traumatic injury, a potentially injurious environment and infection). It promotes escape behaviors, reduces the use of the injured site, and teaches us how to avoid life-threatening events, all of which contribute to survival advantage. By contrast, chronic or persistent pain is maladaptive, evokes human suffering and decreases life expectancy [1]. Persistent pain conditions, such as fibromyalgia syndrome (FMS), temporomandibular joint disorder (TMJD; see Glossary) and persistent lower back pain (LBP), represent some of the most common human clinical conditions; however, our ability to satisfactorily manage these conditions is inadequate [2].

The duality of pain of being consciously avoided and evolutionary necessary creates additional layers of complexity for studying and understanding of pain conditions at all levels, including the genetic level. However, identifying specific genetic elements of pain perception promises to be one of the key elements for creating novel and individualized treatments that will reduce suffering from persistent pain conditions. In this review, we focus on our emerging understanding of the genetic basis of human pain. Some of the most recent discoveries, such as the recognition of the crucial role of voltage-gated sodium channel Nav1.7 in pain perception [3–5]), are outstanding examples of recent contributions to the exponentially developing field of pain. Another example includes the identification of a new mechanisms of polymorphism-dependent gene expression, such as regulation of translation by interacting alleles in the *catechol-O-methyl transferase (COMT)* gene locus [6]), which has contributed to our understanding of the genotypic structure of pain-related genes. In addition, we discuss the evolutionary context of human pain conditions.

Glossary

Allodynia: Pain perception evoked by a stimulus that under normal conditions evokes non-painful sensations.
Balancing selection: A form of natural selection that maintains genetic variation at an individual locus. Unlike positive selection, balancing selection increases levels of nucleotide variation at linked sites. A variety of mechanisms can result in elevated levels of polymorphism including over-dominance (heterozygote advantage), frequency-dependent selection, and temporally or spatially variable selection.
Central sensitization: A phenotypic change in CNS pathways that leads to the augmentation of the processing of nociceptive stimuli.
Comorbid conditions: Medical conditions that occur with high frequencies with other medical conditions.
Fibromyalgia syndrome: This is a chronic musculoskeletal pain condition that is characterized by widespread pain throughout the body. It is associated with increased sensitivity to painful stimuli and psychologic distress. This condition has a prevalence of 3% in the US population and impacts female to males in a 3:1 ratio.
Haplotype: A haploid genotype for multiple alleles at multiple linked loci that are transmitted together.
Hyperalgesia: Enhanced pain perception evoked by a stimulus that under normal conditions evokes painful sensations.
Intermediate phenotype: A quantifiable phenotype that is a specific component or construct associated with a complex medical condition or syndrome. These are measurable traits or markers that contribute to the susceptibility or manifestation of a disorder or condition.
Linkage disequilibrium: The strength of association between alleles at two different markers (pairwise LD).
Nociception: Pain and pain behaviors evoked by the application of a brief noxious stimulus.
Nociceptors: Primary nerve fibers that respond to tissue injury or stimuli that are capable of evoking tissue injury.
Temporomandibular joint disorder: This is a common musculoskeletal pain condition associated with the temporomandibular joint and surrounding muscles of mastication. It is associated with increased sensitivity to painful stimuli and psychologic distress. This condition has a prevalence of 10% in the US population and impacts female to males in a 3:1 ratio.

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Human clinical pain phenotypes: biology or psychology?

A common feature of human pain conditions is that they are multifactorial and present clinically as a mosaic of biologic and psychologic phenotypes [1]. This is particularly true of idiopathic pain disorders (IPDs), which include the most prevalent pain conditions such as TMJD, FMS, irritable bowel syndrome, chronic headaches, interstitial cystitis, chronic pelvic pain, chronic tinnitus, whiplash-associated disorders and vulvar vestibulitis. The worldwide prevalence of persistent or chronic pain, most of which consist of IPDs, is 15–20% for adults [7]. World Health Organization surveys show that approximately one in ten primary care patients develop a persistent pain condition within 12 months, and ~50% of those who develop a persistent pain condition do not recover within 12 months [8]. Unlike acute pain evoked by injury, IPDs are often poorly associated with signs of measurable tissue or organ pathology. IPDs commonly aggregate as 'comorbid' conditions and are characterized by a complaint of pain and intermediate phenotypes associated with motor function, autonomic balance, neuroendocrine function and sleep. Although the mechanisms that underlie IPDs are poorly understood, these conditions are characterized by interactions between a state of pain amplification and psychologic distress. Additional difficulties for diagnosis arise from the substantial variability in the relative contributions of pain amplification and psychologic phenotypes to IPDs, which depends on, and is modified by, exposures to environmental events that evoke tissue injury or psychologic distress [1,9].

Are there surrogate models of human pain conditions?

Several animal and human models have been proposed, some of which reproduce the intermediate phenotypes associated with human persistent pain conditions (Table 1). Noxious thermal, mechanical, ischemic and algesic chemicals or irritants can be applied to various anatomic sites to evoke quantifiable verbal and behavioral measures of pain sensitivity in experimental models that mimic aspects of human pain conditions [10,11].

Animal models (which use behavioral, pharmacologic, electrophysiologic, and histologic techniques) have proven extremely useful in studying the mechanisms underlying the neurobiology of pain processing and analgesia. Recently, however, pain researchers have expanded their technical toolbox to include transgenic mice and knockout mice that have been proven to be effective in identifying genes that mediate specific components of pain processing. A recent striking example is the gene encoding the Runt domain transcription factor *Runx1*. *Runx1* is expressed on most nociceptors (sensory pain fibers) during embryonic development but becomes restricted to a smaller population of pain-sensing fibers in adult animals [12]. In these neurons, Runx1 regulates the expression of many ion channels, receptors, and signaling molecules that respond to tissue injury. Mice lacking *Runx1* exhibit diminished responses to both heat and cold thermal stimuli. Additionally, they fail to develop mechanical allodynia after nerve injury. Because Runx1 regulates the expression of nociceptor phenotypes, it provides an attractive target for human association studies and the development of novel pharmacotherapeutics.

A comprehensive catalog of genes implicated in pain and analgesia in mouse models has recently been consolidated in a *Pain Genes Database* [13] (http://paingeneticslab.ca/4105/06_02_pain_genetics_database.asp). Of the ~200 genes implicated in pain sensitivity through mouse knockout studies in this database, we summarized information regarding ~100 genes shown to produce specific noncontradictory alterations in pain behavior(Table S1, Supplemental Material online).

Although animal models have proven extremely effective in teasing apart the underlying biologic processes and signaling pathways that mediate pain perception and behaviors, they are unable to reproduce human pain conditions in their full complexities [14]. Because animal studies are usually conducted on only one or a few inbred strains, results are not always applicable to human pain conditions. Furthermore, it has recently became evident that the genetic structure for alternative splicing events are substantially divergent between humans and mice [15]. Thus, human association studies in clinical populations are required to validate the findings from genetically engineered mice.

Genetic background of human clinical pain phenotypes

The complete inability to sense pain in an otherwise healthy individual is a very rare phenotype. Patients with congenital insensitivity to pain (CIP) have a sensory-discriminative deficit and do not perceive pain, whereas subjects with congenial indifference to pain (CIDP) have loss of the affective-motivational component of pain perception–they can recognize painful stimuli but do not show withdrawal responses [3–6]. Such individuals often die in childhood because they fail to perceive injuries and infections, which reinforces the view of the necessity of pain perception for species survival [3–5,16]. CIP, also referred to as hereditary sensory and autonomic neuropathy (HSAN), is distinguished from CIDP by the presence of peripheral neuropathy. At present, five types of HSAN have been identified, which are caused by mutations in five distinct genes. Although these genes cannot be integrated into one specific pathway, severe mutations in each of these genes lead to a related pain phenotype [16] (Table 2).

Several recent publications have identified the α-subunit of sodium channel $Na_V1.7$ (SCN9A) as an essential and nonredundant requirement for nociception in humans [3–5,17]. After the discovery of Cox *et al.* [3] that three distinct familial nonsense mutations, which cause the loss of function in the $Na_v1.7$ channel, are linked to the inability to perceive any form of pain, Goldberg *et al.* [17] reported that the loss-of-function mutations in *SCN9A* underlies the congenital indifference to pain in multiple human populations. Nine loss-of-function mutations of *SCN9A* have been identified in nine families from seven nationalities. In both reports, affected individuals showed a loss of pain perception, although the ability to distinguish sharp from dull stimuli was preserved, and affected individuals were able to detect differences in temperature. Motor reflexes and autonomic responses were also normal, suggesting an

Table 1. Animal and human experimental pain models

Clinical pain condition	Surrogate animal model	Surrogate human model	Refs
Cutaneous pain			[11,57]
Burn pain	No	UV burn; heat burn; freeze burn	
Postoperative incision pain	Incision model	No	
Cancer pain	Implantation of tumor cells; chemotherapeutic agents	No	[10]
Musculoskeletal			[10,11,58,59]
Muscle pain	Carrageenan; acidic saline; hypertonic saline	Exercise; hypertonic saline	
Fibromyalgia syndrome	Stress-induced hyperalgesia models	Sleep deprivation	
Lower back pain	Lumbar root injury	No	
Arthritis models	Surgical production of a joint defect; Carrageenan; complete Freund's adjuvant[a]; Kaolin[a]; lipopolysaccharide; uric acid crystals	No	
Temporomandibular joint disorders	Carrageenan; CFA	Hypertonic saline	
Primary headache conditions			[60]
Migraine	Vascular models; neurovascular models	Nitroglycerin or calcitonin gene related peptide – jugular vein infusion	
Tension	No	No	
Neuropathic pain conditions			[10,61]
Neuropathic pain	Nerve injury models; rhizotomy; CNS lesions; streptozocin diabetic models; Varicella-zoster virus injection; lysolecithin demyelination	Post-ischemic paraesthesia and/or dysesthesia	
Visceral			[10,62]
Acute visceral pain	Stimulation of hollow organs	Stimulation of hollow organs	
Angina pectoris	No	Adenosine infusion	
Irritable bowel syndrome/colitis	Injection of inflammatory substances: trinitrobenzene sulfonic acid, zymosan	No	
Vulvar vestibulitis	No	No	
Interstitial dystitis	Injection of inflammatory substances	No	

[1]These are inflammatory agents often used in animal model of pain and inflammation.
CFA, complete Freund's adjuvant.

absence of peripheral nerve abnormalities. Although Goldberg *et al.* [17] have referred to this condition as 'congenial indifference to pain' (CIDP; autosomal recessive [16] OMIM 243000, http://www.ncbi.nlm.nih.gov/sites/entrez), Cox *et al.* proposed the term 'channelopathy-associated insensitivity to pain' [3]. Cox *et al.* [3] argued that congenital indifference to pain resulting from mutations in *SCN9A* is a form of insensitivity to pain because the defect is caused by a channelopathy that is not usually detected by routine histopathology. By contrast, mutations in *SCN9A* that lead to excessive channel activity trigger activation of pain signaling in humans and produce a primary erythermalgia (OMIM 133020), which is characterized by burning pain in response to exposure to mild warmth [4]. Mutations in this gene also produce a rare condition referred to as 'paroxysmal extreme pain disorder', which is characterized by rectal, ocular and submandibular glandular pain (OMIM 167400) [4,5].

Mutations in the α_1-subunit of a different voltage-gated sodium channel, $Na_V1.1$ (*SCN1A*), have recently been associated with 'familial hemiplegic migraine Type 3' (FHM3 OMIM 609634; Table 2) [18]. The disorder is characterized by hemicranial migraine attacks and hemiparesis (i.e. the partial paralysis of one side of the body). In addition to FHM3, FHM1 and FHM2 subtypes have been described (OMIM 141500 and 182340) and linked to mutations in the α_{1a}-subunit of a voltage-gated calcium channel (*CACNA1A*) and the α_2-subunit of Na^+/K^+-ATPase pump *(ATP1A2,)* respectively [18].

These discoveries have pinpointed primary genetic candidates (e.g. sodium and calcium channels) that might contribute to more common pain disorders. It is attractive to suggest that polymorphisms in these gene loci that produce quantitative rather than qualitative changes in gene function underlie less severe but more frequent human pain conditions. It has become increasingly evident, however, that most common clinical pain phenotypes are best classified as multifactorial or complex disorders [1] rather than rare mutational disorders because they are induced and influenced by both diverse environmental factors (e.g. trauma, lifestyle and stress) and a complex array of genetic polymorphisms. Although candidate genes that have been identified in linkage analysis studies of mendelian pain disorders might have significant roles in complex pain disorders, many more genetic variants are expected to contribute.

With recent advances in high-throughput genotyping methods, the list of genes associated with persistent pain conditions is rapidly increasing (Table 2). Because of the high comorbidity between clinical pain conditions, it is expected that the identified genes will be implicated in more than one condition. Thus, common polymorphisms in the promoter region of the serotonin transporter (*SLC6A4*) gene are associated with FMS [19], chronic fatigue syndrome [20], migraine headache [18] and TMJD [21].

An important characteristic of clinical pain phenotypes is the range of individual responses to pharmacologic agents. Although individual responses to drugs are affected by age, sex, drug interactions, disease status and environmental stress level, individual genetic variations influence both the efficacy and side effect profiles of drugs used to treat pain conditions. Multiple copies of *CYP2D6* (which encodes cytochrome P-450, a member of family of enzymes that catalyses many reactions involved

Table 2. Genetic variants implicated in human pain conditions[a]

Functional class	Gene[b,c]	Condition	Genetic polymorphism[d]	Minor allele frequency	Refs
Transporters	*SLC6A3, or DAT1*	PTSD	3′ Untranslated region VNTR	0.305	[19]
	SLC6A4, or 5-HTT, or SERT	FMS, CFS, D-IBS	5-HTTLPR	0.42–0.452	[19,20,63]
		Migraine	VNTR and 5-HTTLRP	0.42–0.452	[18]
		TMJD	VNTR in intron 2	0.454	[21]
	ABCB1, or MDR1	Efficacy of mu-opioid analgesia	C3435T silent polymorphism in exon 26 changes substrate specificity.	0.539	[64,65]
Metabolic genes and transcription regulators	*COMT*	TMJD, analgesia, migraine, FMS	Val158Met Three major haplotypes determine COMT activity.	0.25–0.50	[6,39,64, 66–68]
	CYP2D6	Opioid analgesia	More than 75 alleles code for distinct metabolizer phenotypes.	0.001–0.207	[64]
	GCH1	LBP	A haplotype of the GCH1 gene was associated with less pain following diskectomy for persistent radicular LBP.	0.154	[69]
	MTHFR	MA	C677T	0.35	[18]
	ACE	MA	287-bp deletion in intron 16.	0.35	[18]
	SPTLC1, or SPT1	HSAN I	Autosomal dominant mutations Cys133Tyr, Cys133Trp, Val144Asp and Gly387Ala resulted in reduced SPT activity and impaired sphingolipid synthesis.	Rare	[16,70]
	HSN2	HSAN II	Autosomal recessive mutations led to truncated forms of deduced HSN2 protein with unknown function.	Rare	[71]
	IKBKAP, or IKAP	HSAN III	Mutation in intron 20 produced truncated protein. Arg696Pro in exon 19 disrupted phosphorylation.	Rare	[72]
	NGFB	HSAN V	Autosomal recessive mutation Trp211Arg	Rare	[73]
Receptors	*NTRK1, or TRKA*	HSAN IV	43 known autosomal recessive mutations led to inability to transduce NGF into growing neurons and their death.	Rare	[74]
	ADRA2A, ADRA2C	C-IBS	α_{2C}Del322–325 and α_{2A}C-1291G polymorphisms were associated with constipation-predominant IBS.	0.08, 0.43	[63]
	ADRB2	TMJD	3 major haplotypes were identified.		[36]
	DRD2	MA	His313His (*NcoI* restriction site polymorphism C/T)	0.27	[18]
		PTSD	3′-UTR polymorphism	0.38	[19]
	DRD4	MO, FMS	Exon III tandem repeat polymorphism	0.104–0.5	[19,75]
	MC1R	Opioid analgesia	Arg151Cys, Arg160Tyr and Asp294Asn	0.020–0.045	[26,27]
	OPRM1, or MOR	Mu-opioid analgesia	A118G (Asn40Asp)	0.172	[27,64,76]
	5-HTR2A	MA,TMJD	T102C	0.445	[68,77]
Cytokines	*IL1A, IL1B, and IL1RN*	LBP	IL-1α C889T, IL-1β C3954T, and IL-1RN G1812A	0.29, 0.21, 0.17	[78]
	IL1RN	VVS	Second intron VNTR	0.482	[79]
	IL1B	VVS, BMS	C3954T	0.25	[80,81]
	IL6	LBP	T15A (Asp162Glu)	0.015	[82]
	IL10	IBS	Promoter polymorphism G-1082A	0.48	[63]
	TNF	IBS	Promoter polymorphism G-308A	0.17	[63]
	LTA	MO	A higher frequency of TNFB*2 allele in MO patients.	0.614	[75]
Ion channels	*ATP1A2*	BM	Arg548His mutation was found in BM family.	Rare	[18]
		FHM type 2	Missense mutations and deletions led to loss of function or alteration of the kinetics of Na^+/K+-ATPase pump.	Rare	[18]
	CACNA1A	MA	Significantly increased sharing of CACNA1A marker alleles in siblings with MA.		[18]
		FHM type 1	Missense mutations in a pore-forming α1A subunit of neuronal $Ca_V2.1$ channels.	Rare	[18]
	SCN1A	FHM type 3	Gln1489Leu mutation led to a faster $Na_V1.1$ channel recovery from fast inactivation.	Rare	[18]
	SCN9A IL6, or IFNB2	PE	Mutations enhanced activation of $Na_V1.7$ channels.	Rare	[4]
		PEPD	Mutations impaired inactivation of $Na_V1.7$ channels.	Rare	[5]
		CAIP, or CIDP	Homozygous nonsense mutations caused loss of function of $Na_V1.7$ channels.	Rare	[3,17]

[a]Abbreviations: BM, basilar migraine; BMS, burning mouth syndrome; CAIP, channelopathy-associated insensitivity to pain; CIDP, congenital indifference to pain; CFS, chronic fatigue syndrome; FHM, familial hemiplegic migraine; FMS, fibromyalgia syndrome; HSAN, hereditary sensory and autonomic neuropathy; IBS, irritable bowel syndrome; LBP, low back pain; M6G, morphine-6-glucuronide; MA, migraine with aura; MO, migraine without aura; NGF, nerve growth factor; OA, osteoarthritis; PE, primary erythermalgia; PEPD, paroxysmal extreme pain disorder; PTSD, posttraumatic stress disorder; TMJD, temporomandibular joint disorder; VA, vasospastic angina; VNTR, variable number tandem repeat; VVS, vulvar vestibulitis syndrome; 5-HTTLPR, 5-HTT gene-linked polymorphic region.
[b]Gene abbreviation is given in accordance with the NCBI *Gene* database.
[c]Direction of association between genetic variant and pain phenotype.
[d]Original references are included in Table S2 in the online supplementary material.

in drug metabolism), are associated with enhanced responses to the analgesic codeine, which can increase its toxicity by increasing the metabolism of codeine to morphine [22]. By contrast, codeine and tramadol (another opioid analgesic) are ineffective in patients with genetic variants that result in low or no CYP2D6 activity [23,24]. More than 75 *CYP2D6* alleles have been identified, ranging from nonsynonymous mutations to single nucleotide

polymorphisms (SNPs) that either alter RNA splicing or produce deletions of the entire gene [25].

Another striking example of genetic-dependent individual variations in drug response has been provided by Mogil *et al.* [26]. The authors showed that nonfunctional variants of the melanocortin 1 receptor (*MC1R*), which produces a red hair and fair skin phenotype, were associated with an increased analgesic response to κ receptor–mediated opioid analgesia. Red-headed women required less of the κ-opioid pentazocine to reach a specific level of analgesia compared with all other groups [26]. This study presented the first strong evidence for a gene-by-sex interaction in the area of pain genetics, because the authors also showed that red-headed men did not experience enhanced κ-opioid analgesia [26]. This discovery was preceded by animal studies that showed that *Mc1r* mediates κ-opioid analgesia in female mice only. By contrast, the analgesic effects mediated by morphine-6-glucuronide (M6G), a metabolite of morphine that is selective for μ-opioid receptors, did not produce sex-specific analgesia. Red heads of both sexes displayed reduced sensitivity to noxious stimuli and increased analgesic responsiveness to M6G [27]. These examples strongly suggest that using high-throughput clinical tests will soon make it possible to individualize therapies based on genotypic data in a manner that maximizes drug responses and minimizes side effects and morbidity.

The identification of an increasing number of genetic variants associated with clinical pain phenotypes is in line with the multifactorial nature of these phenotypes. Therefore, it is expected that there are many genetically modulated pathways that impact the onset and maintenance of common clinical pain conditions, which vary in clinical profile and associated signs, symptoms and comorbidities.

Multiple pathways of vulnerability: evolutionary advantage or modern-day headache?

Why are persistent pain conditions that are very common in the population and that have a substantial genetic component maintained at relatively high frequencies in the population and not eliminated by natural selection? Two major hypotheses have been proposed to explain this phenomenon: the common-disease/common-variant (CDCV) hypothesis and the common-disease/rare-variant (CDRV) hypothesis [28–30]. The CDCV hypothesis proposes that complex traits result from underlying genetic variants that occur with a relatively high frequency, but the effect size of each of the variants is relatively small [28,29]. The penetrance of each of the variants is low, because it requires an interaction with many other specific genetic variants and particular environmental conditions [1]. By contrast, the CDRV hypothesis states that complex traits have numerous underlying genetic variants, each occurring with a low frequency in the population but with high penetrance.

Credible arguments have been generated by advocates of both hypotheses [28–30], and specific examples have been reported (Table 2). Here, we propose that both rare deleterious genetic variants and common genetic polymorphisms underlie human pain perception and clinical pain phenotypes (Figure 1), which embraces the theoretical and practical arguments associated with both hypotheses. Although the model can be applied to any common disorder, we discuss it in terms of human pain conditions.

We assume that within each clinical pain phenotype there is a proportion of cases attributable to rare, deleterious familial mutations and a proportion of cases attributable to a combination of common genetic variants in the population. A significant number of the most severe cases are caused by rare familial deleterious mutations in genes encoding crucial components of pain pathways. The penetrance of these deleterious mutations is high, and the relative contribution of environmental factors to the onset of these cases is minimal. Genotyping SNPs that commonly exist in this population will not identify or classify the condition. However, specific observable intermediate phenotypes (such as pain sensitivity or anxiety), should be informative and substantially different from the populations mean values for these measures.

Examples of mendelian diseases, where causal genes show broad allelic diversity [28], provide a strong argument for the possibility that rare alleles contribute to complex human diseases. However, the contribution of

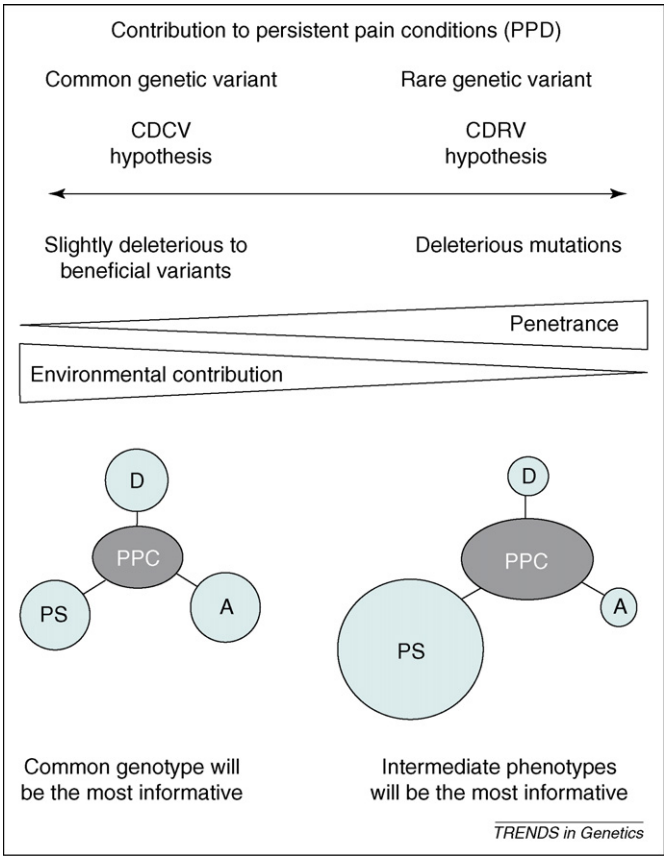

Figure 1. Disease allele frequency in common persistent pain conditions (PPCs). This model assumes that within each clinical pain phenotype, there is a proportion of cases that are attributable to rare deleterious mutations and a proportion of cases attributable to a combination of common genetic variants in the population. A significant number of the most severe cases are caused by rare, high-penetrance familial deleterious mutations. Within these cases, at least one of the observable intermediate phenotypes, such as pain sensitivity (PS), depression (D) or anxiety (A), will differ substantially from the mean values in the population. On the other hand, there are cases underlined by an interactive array of low-penetrance genetic variants that are common in the population. Disease onset of these cases will be driven by environmental pressures and the mean values for many of the associated intermediate phenotypes will be slightly increased.

these rare deleterious mutations to the onset of common diseases can be significant only if (i) the frequency of these disease-predisposing genetic variants is relatively high in population; (ii) a sufficient proportion of *de novo* mutations in humans are mildly deleterious; and (iii) the intensity of selective pressure against them is reasonably weak [30]. Kryukov *et al.* [30] have estimated that the prevalence of mildly deleterious new missense mutations is ~53%. Up to 70% of the overall low-frequency missense alleles are mildly deleterious and are associated with a heterozygous fitness loss of 0.001–0.003. It is unlikely that missense mutations can account for the diversity observed in persistent pain conditions, but they might contribute to the manifestation of the most extreme pain phenotypes, as has been recently observed for obesity [31].

On the other end of the proposed model is the proportion of clinical pain phenotypes that are caused by a combination of genetic variants. Each genetic variant can be slightly deleterious or even beneficial for other traits and can contribute to a clinical pain phenotype when it interacts with another variant (Figure S2, Supplemental Material online) or after exposure to various environmental factors (i.e. injury or stress). In this situation, intermediate phenotypes, such as pain sensitivity and anxiety, will be less informative because each of the intermediate phenotype will not deviate dramatically from the population means; however, well-designed case-control studies with reasonable sample size that examine common genotypes might yield valid associations.

The proposed model is in agreement with the prediction published by Roberson in 1967 [32]. He suggested that the effect size of functional genetic variations should be exponentially distributed, with a few variations of major effect and increasingly more with smaller effects. Experimental evidence for the contribution of multiple interacting loci with different effect size on complex traits has been supported by several *Drosophila melanogaster* mapping studies. For example, 53 quantitative trait nucleotides (QTNs) affect the number of mechanosensory bristles [33]. The effect size of each QTN was clearly exponentially distributed, and the effects of most QTNs were conditional on sex (27%), temperature (14%) or both sex and temperature (30%). Epistatic interactions between QTNs were also common [33].

There has been an exponential increase in the number of association studies that have identified genotypes that are associated with a variety of highly prevalent diseases, providing conformation for the CDCV hypothesis. Given these observations, one must ask why such polymorphisms are medically detrimental but not evolutionary deleterious? Three explanations have been proposed [28,30]. First, a late-onset disease does not affect reproductive fitness. This mechanism is least applicable for chronic pain conditions, because a substantial proportion of these conditions impact women during their reproductive age [1,34]. Second, an increased level of persistent 'psychologic stress', derived from social expectations, has created new environmental pressures that serve as a strong driver for persistent pain conditions. Although this is plausible and an attractive explanation; there is substantial evidence that many diseases, including migrainous headache, existed in human populations for a long time [34] (the first written description are found in the Medical Papyri of Ancient Egypt at ~1500 B.C. [35]). Finally, a significant contribution of balancing selection seems probable, and there are a few examples that are consistent with a balancing selection mechanism [36,37]; however, further proof is required. Among plausible mechanisms of balancing selection, a 'trade-off' mechanism—where the negative effect of the genetic variant on one trait is compensated by its positive effect on another—might explain why genetic variants underlying human clinical pain conditions persist in the population [37] (Figure S1, Supplemental Material online).

Thus, although individuals expressing severe pain conditions might be considered disadvantageous, these individuals are also characterized by a state of elevated somatization—an increased awareness of bodily sensations [38]. In other words, these individuals have an increased capacity to sense essentially all environmental stimuli, such as light, noise and odor, which arguably increase their ability to detect and avoid environmental threats. From this point of view, lower pain threshold, at least at some level, is commonly associated with the ability to detect a wide variety of non-noxious sensory stimuli. Furthermore, individuals who are prone to develop TMJD, a facial chronic facial pain condition, have lower pain thresholds and higher somatization scores before the development of the conditions [39,40]. If these pain conditions develop after exposure and if these exposures are avoided during a life time, individuals should benefit from the advantage of having high sensory acuity without the disadvantage of experiencing a persistent pain state.

Most IPDs have a higher female prevalence, which is consistent with substantial evidence that, as a population, women are more sensitive to noxious stimuli than men. Women also show a generalized increase in somatization (vigilance) and sensory acuity compared with men [41]. Fillingim and Maixner [41] proposed that sex-dependent differences in pain sensitivity results from evolutionary pressures on pain regulatory systems that promoted greater sensory acuity in women, which permits women a greater awareness of the immediate environmental threats resulting in a greater protection of offspring. In women, strong pain-suppressing responses through endorphins and catecholamines are evolutionarily detrimental because endorphins and catecholamines, impair ovulation and thus negatively impact reproductive potential [41,42].

Haplotypic diversity: effects on pain phenotypes

If a significant proportion of clinical pain phenotypes are caused by relatively frequent genetic variants, what is the pattern of genotypic diversity? It is tempting to assume that a strong functional polymorphism dominates within each gene locus that can be slightly modified by other secondary polymorphisms within the gene locus. This situation is similar to what is observed for familiar deleterious mutations that cause mendelian diseases, where there is a strong and easily measurable effect on the associated phenotypes that is proportional to the number of copies of the disease-related genetic variant [29,30]. However, there is increasing evidence that supports the view that the genotypic architecture of the

human genome is much more complex than currently appreciated. Multiple functional interacting loci within one gene locus are likely to contribute to the same or related pain phenotypes, which complicates the analysis and interpretation of association study results.

For example, one gene, catechol-*O*-methyltransferase (*COMT),* encodes an enzyme that metabolizes catecholamines (e.g. dopamine, norepinephrine and epinephrine) and thus affects an array of cognitive-affecting phenotypes, including pain phenotypes [39,43–45]. The functional *val158met* polymorphism within the *COMT* locus was one of the first examples of a common genotype that affects gene activity [46] Substitution of Val to Met at codon 158 of *COMT* results in a threefold reduction in thermostability and activity of the enzyme [46]. However, during the past decade, several new functional polymorphisms were identified. [6,37,39,44,47,48]. Thus, a haplotype combining the $val^{158}met$ (rs4680) polymorphism with two other common SNPs, one upstream in intron 1 (rs737865) and another in the 3′ untranslated region (rs165599), is highly associated with schizophrenia [47] and differentially affects the expression of rs4680 alleles in human brain tissue [49]. Another potentially *cis*-acting functional SNP (rs2097603) has been located upstream of the promoter of *COMT* that drives transcription of the predominant form of membrane-bounded (MB) *COMT* in the brain. This variant also affects COMT activity in lymphocytes and postmortem brain tissue [37]. Furthermore, three common haplotypes of *COMT*, consisting of two synonymous (rs4633 and 4818) and one nonsynonymous $val^{158}met$ SNPs, resulted in a 30-fold difference in enzymatic activity and are associated with pain sensitivity. Protein translation is regulated by an interaction between three alleles in these haplotypes, which creates distinct secondary structures of the corresponding mRNAs [6]. Furthermore, $val^{158}met$ and the SNPs in the promoter are linked to affective-cognitive phenotypes, whereas the three haplotypes in the coding region of the gene are strongly associated with sensory-discriminative aspect of pain [44,48].

Thus, *COMT* contains at least five functional polymorphisms that impact its biologic activity and associated phenotypes. The potentially complex interactions of functional variations in *COMT* imply that the overall functional state of the gene might not be easily deduced from genotype information alone, which presumably explains the inconsistency in the results from association studies that focus on the $val^{158}met$ polymorphism [50]. This complex genotypic pattern can be explained by the presence of a single balanced polymorphism in the *COMT* locus such as the $val^{158}met$ amino acid variation distinguishing Val158 and Met158 alleles (Figure S1, Supplemental Material online) or stable combinations of several compensatory changes tightly linked to a single balanced polymorphism. Levels of neutral or compensatory polymorphisms are expected to be elevated when these polymorphisms are tightly linked to a balanced polymorphism, and the effect can be large under certain population parameters. Apparently, it is tightly linked polymorphic regions that natural selection holds in the population and not just the balanced polymorphism [51].

Another explanation for a high level of functional haplotypic complexity of the *COMT* gene locus relates to the pleiotropic complexity of COMT function that results in multiple phenotypes that vary in cognitive-affective and sensory-discriminative capabilities. One of the best-developed illustrative examples comes from studies on *D. melanogaster* that examined the functional allelic variation in the catecholamines up (*Catsup*) gene, which encodes a negative regulator of tyrosine hydroxylase, the rate-limiting step in the synthesis of catecholamines. Although *Catsup* is a pleiotropic quantitative trait gene, most individual QTNs do not have pleiotropic effects, and molecular genetic analyses of *Catsup* sequences are consistent with a balancing selection that maintains multiple functional polymorphisms [52]. This conclusion is easily reached from studies on *D. melanogaster,* where individual polymorphisms show very little linkage disequilibrium (LD) [52]. In humans, polymorphic variants within one gene locus very often are not independent and form haploblocks, where SNPs exhibit high LD [53]. In this situation, functional genetic variants within one gene may influence each other substantially, especially if the affected phenotypes are not independent, as is the case for different modalities of human pain perception [48].

Analysis of genotyping results for pain phenotypes: current challenges and future prospective

In common with other complex traits [34,35], there are several levels of genetic complexity underlying human clinical pain phenotypes. First, such phenotypes represent an interactive array of multiple nonindependent intermediate phenotypes. Second, that intermediate phenotypes are shaped by multiple interacting genes and environmental exposures, most of which have small individual effects on the condition of interest. Third, many of contributing loci are likely to possess complex genotypic and haplotypic structures with epistatic interactions within single gene locus.

Thus, adopting the principles and methods that have proven effective with simpler mendelian traits, which show linear relationships between the measured trait and genotypic variability at one gene locus and that assumes context invariance, fails to explain common pain phenotypes. Furthermore, the analytic strategies that are typically used today make the implicit assumption that interacting loci can be identified through their independent and marginal contributions to trait variability. However, if interacting loci are frequent, and the effect of the interaction is substantial, the power of current statistical approaches to detect functional polymorphisms will be a substantial barrier [29]. We are thus dependent on the development of new analytic methods and computational strategies that will enable the identification of combinations of variable and interactive loci at a genome-wide level to account for epistatic effects on quantitative pain traits [54–56].

Another challenge for the genetic pain field is that some clinical pain conditions that are caused by rare hereditary mutations, which are carried by specific individuals, will escape detection by genotyping methods for polymorphisms that commonly exist in the population. Until affordable high-throughput sequencing methods arrive, which

permit sequencing of each patient for everyday use, the identification of measurable and informative clinical intermediate phenotypes can serve as good proxies for rare mutations that have a strong effect size. Thus, for practical reasons, the assessment of both intermediate phenotypes and common risk genotypes will be the most informative in subclassifying chronic pain patients. Given the exponential successes in the area of pain genetics over the past few years, it is realistic to assume that evidence-based genetic approaches will be developed in the near future for tailoring of individual treatments and therapies for common clinical pain conditions.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tig.2007.09.004.

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