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Genetic Contributions to Clinical Pain and Analgesia: Avoiding Pitfalls in Genetic Research

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Abstract

Understanding the genetic basis of human variations in pain is critical to elucidating the molecular basis of pain sensitivity, variable responses to analgesic drugs, and, ultimately, to individualized treatment of pain and improved public health. With the help of recently accumulated knowledge and advanced technologies, pain researchers hope to gain insight into genetic mechanisms of pain and eventually apply this knowledge to pain treatment.

Perspective—We critically reviewed the published literature to examine the strength of evidence supporting genetic influences on clinical and human experimental pain. Based on this evidence and the experience of false associations that have occurred in other related disciplines, we provide recommendations for avoiding pitfalls in pain genetic research.

Keywords

Genetic association study; single nucleotide polymorphism; haplotype; population stratification; multiple test correction

Exploring the genetic basis of human variations in pain is vital to understanding the molecular basis of pain sensitivity, variable responses to analgesic drugs, and individualized treatment. Recent knowledge and advanced technologies have helped pain researchers gain insight into genetic mechanisms of pain and apply this knowledge. Several recent publications suggest that specific genetic variations produce inter-individual differences in pain sensitivity and analgesic drug responses.^{15,45,60,85,97}

However, the influence of genetic factors in human pain is generating a mixed picture. Considering the complexity of pain biology and the size of the human genome, it is not surprising that variable study designs, sample heterogeneity, small sample sizes, phenotype complexity, and alternative statistical approaches can result in inconsistent or even opposite outcomes. Using appropriate methodologies is therefore critical, as exemplified by sex-related genetic association claims that have been subsequently questioned as they often lack adequate internal and external validity.⁶⁸

An example from the pain literature illustrates how methodologic variability can lead to conflicting results. An initial report based on 29 subjects suggested that a single nucleotide polymorphism (SNP) of catechol O-methyl-transferase (COMT) gene inducing amino acid change at codon 158 from valine to methionine (COMT val158met) significantly increased experimental pain responses.⁹⁷ Though intriguing, independent follow-up studies of this

association have reported conflicting results. While the association was replicated in an experimental temporal-summation model but not in a phasic-pain model in 1 study,¹⁵ other studies failed to replicate regardless of the pain models.^{14,37} The opposite influence has also been reported for this SNP as patients with the methionine allele require a lower amount of morphine compared to those with the valine allele to treat cancer pain.^{71,72} This reversed association, so called “flip-flop” phenomenon, can arguably and hypothetically suggest confirmation of the previous findings, though it can easily be explained that differences in genetic background, environment, or linkage disequilibrium (LD) can cause it.⁵¹ Moreover, a recent study using a similar cancer pain condition found that *COMT* polymorphisms affect central side effects of morphine but not pain or the amount of morphine requested.⁷⁴ Mixed results for *COMT* using 2 different ethnic populations were also recently reported, supporting the potential effects of sample selection in the genetic association studies.⁹⁰ While these disparate findings could reflect biological variability across clinical and experimental conditions, a lack of methodological consistency among pain researchers may also contribute to nonreplicable associations. The most common causes of a failure to replicate genetic-association findings are in-sufficient statistical power, population stratification, or various forms of between-study heterogeneity (including pain models) or environmental influences. In addition to those traditional causes, it has also been suggested that the strength of a genetic effect can vary by age, causing age-varying associations.⁴³

We critically reviewed the published literature to examine the strength of evidence supporting genetic influences on clinical and human experimental pain. Four PubMed searches as (1) “Pain/genetics”[Majr] Limits:Humans, (2) (“Pain/physiopathology”[Majr] OR “Pain/therapy”[Majr] OR “Pain Threshold/physiology”[Majr]) AND “Enzymes and Coenzymes”[Majr] Limits:Humans, (3) (pain[ti] OR “Pain/physiopathology”[Majr] OR “Pain/therapy”[Majr] OR “Pain Threshold/physiology”[Majr]) AND “Enzymes and Coenzymes”[Majr] AND (Genetics[Majr] OR genetics[-subheading] OR genetic[ti] OR genetics[ti] OR genetically[ti]) Limits:Humans (4) pain[ti] AND (genetic[ti] OR genetics[ti] OR genetically[ti]) Limits:Humans, generated a list of 313 articles for the last 5 years through November 2008. Excluding rare mutations or diseases, reviews, case reports or unrelated articles, the published findings of the genetic influences on pain in humans is summarized in Tables 1 to 4, grouped by clinical pain conditions and experimental pain paradigms. Genetic association studies in chronic clinical pain conditions are summarized in Table 1 and 2, while those of surgically induced acute clinical pain are summarized in Table 3, and experimentally induced pain studies are in Table 4. Studies are summarized based on the critical study design factors in genetic research, ie, sample size, population stratification, measured phenotype, and statistics used, followed by the results and conclusion. Few genome-wide association studies (GWAS) have been reported for pain, so most of these represent candidate gene association studies based on a priori assumptions of the relevance of the candidate gene to pain perception, transmission, or its modulation. The genetic polymorphisms studied pertain to only a very small percentage of the estimated number of genes in the human genome, approximately 20,000, and a trifling proportion of the total estimated 13 million SNPs.

While experimental pain remains the most common way to accumulate significant numbers of subjects for genetic association studies (14 papers reporting on 9 genes), several studies have evaluated more complex clinical-pain conditions such as cancer pain (8 papers) and low-back pain due to sciatica and disc displacement (9 papers). It should be noted that these tables are still quite simplified. Detailed methods on how subjects were challenged should also be considered in the interpretation. Even in 1 genetic association study of experimental pain in the same cohort, the *COMT* val158met was significantly associated with tonic-pain model (rate of temporal summation of heat pain) but not with phasic-pain models (thresholds and tolerance to thermal, ischemic and mechanical stimuli).¹⁵ Therefore,

generalization based on these publications is limited by the lack of replication in the study designs, even for closely related outcomes for the same gene with the same patient cohort in low-back pain.^{59,85} Recent reviews on the genetic studies of pain summarize multiple genetic associations in various types of pain based on published reports but generally have not addressed discrepant findings or lack of consistency in the experimental designs.^{17,52}

It is too early to apply these findings in the clinical setting yet. Based on this evidence (summarized in Tables 1 to 4) and the experience of false associations that have occurred in other related disciplines, we provide recommendations for avoiding pitfalls in genetic research of pain. This review addresses specific methodological aspects of the available studies on the genetic contributions to pain and analgesia in order to suggest directions for future studies. Conducting future pain and analgesia genetic studies using rigorous methodologies will reduce inconsistencies and facilitate progress. The remainder of this article will describe some of the more commonly encountered pitfalls in genetic research and will make recommendations for optimal study design. Findings of genetic association studies are not readily applicable to clinical pain and analgesia until enough knowledge is accumulated based on studies following these recommendations and other principles of genetic research.

Pain as a Phenotype

What We Have Learned from Animals

Heritability studies in animals provide strong evidence for the heritability of pain and analgesia-related traits. In a series of inbred-mice studies, Mogil et al⁶² tested up to 12 inbred-mouse strains using several common measures of nociception including thermal, mechanical, and chemical nociception. Neuropathic and inflammatory hypersensitivity in inbred mice were also measured. They demonstrated clear strain differences in each assay, with 1.2- to 54-fold ranges of nociceptive sensitivity. All nociceptive assays display moderate-to-high heritability ($h^2 = .30-.76$) mediated by a limited number of apparent genetic loci. Clusters of pain-related traits have been derived from genetic studies on mice which may share common sets of genes controlling the traits within the clusters.⁴¹ These results suggest that there are at least 5 genetically distinct fundamental types of nociception and hypersensitivity: (1) baseline thermal nociception; (2) spontaneous responses to noxious chemical stimuli; (3) thermal hypersensitivity; (4) mechanical hypersensitivity; and (5) afferent input-independent hypersensitivity.^{41,61,62} Based on these animal studies, pain sensitivity seems to be genetically influenced but pain responses to different types of stimuli may have only partially overlapping or even non-overlapping genetic underpinnings.

Variations in genes that regulate nociceptive processing at several levels of the nervous system may influence the perception of several types of noxious stimuli. Convergence of nociceptive processing across noxious modalities at the cellular level (eg, responses to nociceptive specific and wide-dynamic-range neurons)¹⁹ and the encoding of various forms of noxious stimuli within common supraspinal sites provide both cellular-and systems-based mechanisms by which a genetic polymorphism in 1 gene may influence the coding and processing of several modes of noxious stimuli.²³ It is, therefore, quite plausible that there may be a set of genetic variants that influence the perception of pain across several noxious modalities. Nonetheless, the magnitude and the mechanisms of involvement of those genes are uncertain with our current knowledge but likely vary across noxious stimuli.

Animal models have been successfully extrapolated to human models, and have been useful in generating testable hypotheses. Specific prediction of a pain phenotype in humans based on animal models may be possible as demonstrated for *MC1R*.⁶⁰ In these translational studies, both *MCR1*^{-/-} mice and human redheads displayed reduced sensitivities to noxious

stimuli. Moreover, several papers describe the discovery of genes linked to analgesic responses or adaptations to analgesic administration using murine models.^{41,81} However, while meaningful genetic relationships have been gleaned from animal studies,⁴¹ there is no a priori reason that genetic associations present in rodent pain models will always be reproduced in related human pain states; that is, the contributing genetic factors in human pain states may be distinct. Regardless of this limitation, animal models are very useful for comparative genetics since key-genes coding for critical components of pain and analgesia pathways can be identified.

Pain Models in Humans

In humans, researchers can investigate the role of genetics in pain using 3 general approaches: experimental pain models; carefully controlled clinical-pain states that serve as a model to study acute pain, eg, oral surgery; and observations from disease states, eg, cancer or sciatica. However, this classification does not provide a perfect tool for genetic association studies. As mentioned earlier, specific experimental-pain paradigms are likely controlled by at least partially distinct nociceptive mechanisms based on the pain-evoking stimuli. These distinct mechanisms may be influenced by different sets of genes. For example, tonic pain induced by infusion of hypertonic saline into muscle may have different characteristics compared to phasic-muscle pain in response to short-term electrical stimulation. It is critical to define phenotype clearly and precisely since even subtle differences can activate completely different pathways of pain physiology.^{44,48,86}

Experimental pain is, however, very well-defined, and the subjects can potentially be carefully selected and profiled to determine their basal pain sensitivity, psychophysical rating ability, and to differentiate sensory and affecting components of the pain experience.^{11,12,22,35} Some types of experimental pain have been reported to be predictive of clinical-pain states,^{6,67} despite differences in the intensity and duration of changes due to tissue injury and inflammation, and plasticity in the nervous system associated with chronic pain. Arguably, experimental pain is limited as a surrogate for clinical pain as the precise relationship between experimental-pain and clinical-pain experiences is not clearly established.³⁴

Acute inflammatory pain due to controlled, replicable tissue injury (oral surgery) in otherwise healthy young adults is one of the simpler clinical pain states. Local gene expression profiling indicates, however, that hundreds of different molecules are involved over the first 48 hours after oral surgery and likely contribute differentially to the pain phenotype.⁹³ Added to the genetic factors influencing the expression and function of local mediators are genetic influences on nociceptive-signaling pathways, CNS plasticity, mechanisms influencing mood and gender-related mechanisms. Considering the complexity of pain processing, there are certainly potential pitfalls in genetically comparing pain states like lower-back pain to brief clinical and/or experimental pain stimuli. Even in acute postsurgical pain, it is likely that different molecules are involved in minor surgery such as 3rd molar extraction compared to major surgery. Gene-expression profiles change significantly over a relatively short period of time (immediately after the surgery and at 3 hours) as well as longer period of time (24–48 hours postsurgery).^{45,93}

Disease-Associated Pain

The pathophysiology of more complex forms of tissue damage resulting in chronic pain (eg, lower back pain, fibromyalgia) strongly involve affective components such as anxiety, depression, and suffering, and are not precisely comparable to brief acute clinical- and/or experimental-pain stimuli. It is difficult to collect standardized data from homogenous populations with clinical pain, and the results can easily be biased by unidentified factors.

Nevertheless, many diseases in which pain is the defining clinical feature are strongly heritable. Heritability accounts for approximately 50% of migraine,⁴² 55% for menstrual pain,⁸⁸ 35–68% for low back and neck pain,⁵⁵ 50% for shoulder and elbow pain,³¹ and 40% for the pain experienced in carpal tunnel syndrome.²⁹ It should be noted that the heritability estimate of the incidence of painful conditions such as migraine tends to be higher than the heritability estimate of pain intensity itself as a symptom. Many genetic studies are performed on the diseases and not on symptoms of diseases, such as pain. Lastly, very little study has been devoted to following the trajectory of pain in a disease state independent of the pathophysiology of the disease itself. For example, studies directed at identifying genetic factors controlling inflammatory activity in rheumatoid arthritis over time may not help understand the level of pain, as pain and inflammation are somewhat independent in this disease.⁷

A contribution of genetic factors to painful disease states is not universally observed. Temporomandibular joint (TMJ) pain, for example, was not found to be heritable in 1 twin study: Monozygotic (MZ) twins were no more similar than dizygotic (DZ) twins in reporting painful TMJ symptoms. Reared-together MZ twins showed the same resemblance with reared-apart MZ twins for any TMJ pain-related outcome. This study suggests that either genetic factors or the family environment have a negligible effect on this syndrome.⁵⁸ Even a study of the most well-defined temporomandibular disorder (TMD), TMJ osteoarthritis, has failed to find a significant association with candidate genes.³³ However, a genetic association has been reported in 15 TMD patients among 202 healthy subjects followed prospectively for 3 years.¹⁴ Confounding this problem, the research diagnostic criteria for TMDs are based on clusters of signs and symptoms that may not define a common pathophysiology.

It is even more challenging to find a valid model of neuropathic pain for testing in humans. Even though various types of neuropathic pains are classified together based on symptom clusters for diagnostic purposes, the mechanism of each type of neuropathic pain may involve critically different molecular pathways. The etiologies of many common forms of neuropathic pain are obviously very distinct: traumatic, metabolic, ischemic, toxic, infectious, etc. While this mechanistic uncertainty and variability across clinically distinct neuropathic conditions makes evaluation of genetic influences daunting, the need to explore its biological mechanisms and genetic influences is of clinical importance. Similarly, it is likely that cancer pain is a genetically heterogeneous collection of conditions, and that differs from chronic noncancer pain syndromes.

These examples highlight the need for development of more well-characterized, clinically-relevant models of chronic pain. Such models would be of help not only for genetic research, but to other efforts to study chronic pain mechanisms.

How to Measure a Pain Phenotype in Humans?

Quantified pain phenotypes are very heterogeneous across reports (Tables 1–4) ranging from numerical rating scale (NRS) and visual analogue scale (VAS) to analgesic drug consumption, composite measures of multiple pain scales, and surrogate endpoints, eg, cold-withdrawal time and pain-related cortical potentials. There is no consensus about the best method of measuring pain across phenotypes, and in fact different measures might be more sensitive and reproducible for particular types of pain. The ideal method should: (1) have ratio scale properties; (2) be relatively free of biases; (3) be reliable and generalizable; (4) be simple for patients to use, and; (5) be sensitive to changes in pain.⁷⁰

Visual analogue scales (VAS) have been widely used in pain research for decades and have face validity for providing a monotonic continuous measure of pain sensation. The

objectivity of the VAS for pain measurement has been questioned due to choice of “worst pain imaginable” as the anchor for maximum pain.¹⁸ Rating of pain using a VAS with this anchor can be significantly influenced by the subject’s prior experience. For example, there may be a difference in the “imaginable pain” between women who have endured labor pain and those who have not. Improvement of the VAS has been suggested by replacement of the anchor with common stimuli such as the brightest light ever experienced (for example, the intensity of sunlight when seeing it directly with naked eyes) or the loudest noise.

Data have already been presented suggesting that the overall pain experience may have distinct component pathways and mechanisms, each with its own genetic influences. Additional support for analyzing pain as an aggregate phenomenon is based on principal component analysis (PCA).⁶⁵ Neddermeyer et al performed PCA with 8 pain thresholds and found 3 principal components with eigenvalues > 1. The first component explaining 48% of the total variance carried high loading for all pain stimuli indicating that thresholds shared a common source of variance across stimuli. At the second component (explaining 14% of variance), electrical pain and pressure pain dissociated from cold pain. At the third component (explaining 13%), mechanical pain from von Frey fibers separated further. These results may not justify a composite measure for different pain modalities as they do not provide evidence that the first component explaining 48% of the variance is strictly a genetic component. As described by Kim et al,³⁵ other factors including gender, ethnicity, psychological factors, etc, may have greater influence than genetic variations. The excess margin of the correlation of pressure-pain threshold in MZ compared with DZ twins indicates that genetic factors contribute at most 10% to the variation that is observed.⁵⁴ The portion of trait variance attributable to genetic variation based on sibling correlation for heat and cold pain ranges from .11 to .23.⁴⁰ Considering these mild-heritability estimates of experimental pressure, thermal and cold pain, nongenetic factors have a greater effect than genetic variations. Therefore, it is not surprising that pain thresholds share a common source of variance at the first component and that these factors affect all the different pains in the same manner. After removing these nongenetic effects, weaker factors such as genetic variations can demonstrate influence so that dissociations emerge as in components 2 and 3. Interestingly, the magnitude of component 3 in the observations reported by Neddermeyer et al⁶⁵ is similar to the PCA plot of inbred-animal study concluding that mechanical hypersensitivity and thermal hypersensitivity are genetically dissociable phenomena.⁴¹

Some researchers suggest converting individual pain responses into Z scores and summing them as a unitary-pain score or global-pain ratings, even though a strong correlation between responses to different modalities of nociceptive stimuli should not be expected due to the different neural mechanisms involved.⁵ Z score is calculated as $(X_i - \text{mean})/\text{SD}$ and represents the relative position of the subject in the normal distribution curve of the sample population. Therefore, it may have potential value as a tool for the clinical interpretation of a battery of various quantitative sensory testings into an individual’s global pain profile.⁷³ As a research tool, however, calculation of Z score is based on assuming that the variable follows a normal distribution while some pain modalities do not.^{35,36} Furthermore, aggregation of measures should be avoided until it is known which types of pain are genetically related in humans. On the contrary, animal studies and human studies demonstrate that different painful stimuli induce responses from different genetic networks.^{1,41} It has also been reported that genetic factors vary in their effects on different pain models (ie, tonic or phasic), based on the evoking stimuli.¹⁵ A recent twin study demonstrated that cold pressor pain and contact heat pain are mainly distinct phenomenon from a genetic standpoint.⁶⁶ These findings that genetic influence is largely but not entirely modality specific strongly disagree with such indiscriminate aggregation of pain measures.⁶⁶ Simple mathematical summation of Z scores from different modalities of pain therefore may not necessarily implicate a true functional measure of pain perception.

Another possible objective way of measuring pain is using brain imaging. Functional brain-imaging techniques provide insights into brain activity during painful conditions in comparison to nonpainful conditions. Pain-related activations of the insular, secondary somatosensory cortex regions, anterior cingulate cortex, and prefrontal cortex are reported across subjects and different pain paradigms.⁸ Brain regions such as the anterior cingulate cortex are also thought to participate in the attention network and possibly modulate responses to painful stimuli.⁷⁰ It has been suggested that particular brain areas can be used as an objective indication of pain. However, the question still remains how to interpret the results if a patient's pain report is discrepant with signal intensity in the brain images. Thus, while potentially objective, we may need to understand the meaning of the imaging results better before using imaging results as surrogate endpoints for pain measurements.

As suggested above, there are various methods for measuring pain and they all have different features. Some methods may be more appropriate for the short-duration experimental pain while others are more useful for chronic pain. Some measurements can be applied to clinical pain while others are limited to experimental-pain models. Detailed reviews of the strengths and weaknesses of specific pain assessments for use in clinical trials are available.^{20,89}

Multiple genes with relatively small effects likely influence vulnerability to pain, and there may be no simple correspondence between pain phenotypes and individual genotypes.⁷² Further complicating the discovery of genetic mechanisms of pain and analgesia, genetic variants may cause change in a primary trait such as mood that impacts secondarily on pain.^{21,80} Thus, a gene may not be involved directly in coding for a component of a primary nociceptive pathway in order to be involved in pain. For example, a genetic association study might be performed using 2 populations with and without chronic back pain. A positive association with a gene variant might be due to an effect of that variant directly on a nociceptive pathway, a physiological pathway affecting disease susceptibility or a psychological pathway secondarily affecting the likelihood of experiencing chronic pain.

Genotyping

Single Nucleotide Polymorphisms (SNPs)

One promising approach to test the association of common genetic variations with pain is to evaluate single nucleotide differences among individuals. A DNA variation is defined as a polymorphism if it is sufficiently common in a population, usually higher than 1%. This approach is increasingly practical because 4 million of the estimated 13 million common, single nucleotide polymorphisms (SNPs) are already known. Due to their frequency and distribution, SNPs may serve as superior genetic markers for assembly of a high-resolution map, aiding in the identification of phenotype-related loci.

False findings and misinterpretations can be induced when the allelic distribution of a SNP is skewed. Testing for Hardy-Weinberg equilibrium (HWE) can be helpful to select a good subset of the available SNPs.³ In population genetics, the HWE is the relationship between the frequencies of alleles and the genotype of a population. The occurrence of a genotype stays constant unless matings are nonrandom or inappropriate, or mutations accumulate and the frequency of genotypes and the frequency of alleles are said to be at "genetic equilibrium". If 2 alleles, A1 and A2, exist at the A locus with relative frequencies are p and q , the chance of picking a person at random from a population with A1A1 is p^2 , A1A2 is $2pq$ and A2A2 is q^2 . This simple calculation is the HWE. So far, researchers have tested for HWE primarily as a data-quality check and have discarded loci that, for example, deviate from HWE among controls at significance level $\alpha = 10^{-3}$ or 10^{-4} . Violation of HWE can be caused by nonrandom mating, mutations, selection and migration. Furthermore, many

algorithms for genetic analyses including the haplotype-generating program PHASE are based on the assumption that the SNPs are following HWE. It has been recommended to exclude with caution the SNPs in genetic association studies when they violate HWE since they may not be in HWE in a diseased population.

Even though an SNP is only 1 nucleotide difference, it can influence the function of the product of the gene, the mRNA and protein, dramatically.⁶⁴ Some SNPs in exons alter codons and result in replacement of 1 amino acid by another. In most cases, the replacing amino acid is chemically similar to the original one and the effect of such substitution is minimal. However, SNPs can also result in amino acid replacement by another with a dissimilar side chain and such mutations may reduce the function of the gene significantly.

More than 90% of SNPs are in introns or intergenic regions, and most of them are probably of little functional consequence. However, SNPs in promoter regions can alter the affinity of DNA-binding proteins and modify the level of gene expression. Other SNPs in exon/intron boundaries result in intron retention or exon skipping, thus profoundly changing the structure of the resulting protein. It is also possible that the intron where the SNP is located can function as an RNA interference element.⁷⁵ The existence of SNPs in any portion of genomic DNA can be meaningful for the phenotype because of the complicated dynamics of DNA structure and gene expression (Fig 1). Identification of causal variations, including 3-dimensional structure of genomic or complementary DNA, and SNPs predicting amino acid changes, may be the first step in investigating their genetic role in pain sensitivity. It is then desirable to perform functional tests in animals or in vitro directly measuring the effect of the SNP to confirm the association and to provide a stronger rationale for the relationship to pain sensitivity.^{14,85}

Haplotype

Because recombination will rarely separate loci that lie very closely together on a chromosome, sets of alleles (SNPs) on the same small chromosomal segment tend to be transmitted as a linked block through a pedigree. Such a block of alleles is known as a haplotype. Instead of searching for individual SNPs, the haplotype map focuses on patterns of a few SNPs that define each haplotype. It is thought that the identification of a few alleles of a haplotype, through genotyping of tag SNPs, can unambiguously identify all other polymorphic sites in its block. Such information is collected by the International HapMap Project (<http://www.hapmap.org/>) and contributes to the design of SNP-based microarray platforms. It also helps find a causative SNP of a phenotype in linkage disequilibrium with a SNP of statistical finding. If a specific haplotype is more common among those with a certain phenotype, the polymorphisms linked to that phenotype should be on that same block of DNA.

One problem with defining haplotypes is that block boundaries can vary according to the block definition, the population, and the SNP density. Different algorithms partition haplotype blocks quite differently from each other and there is currently no consensus on the definition that best generates haplotype-block structures. The properties of haplotypes from subjects should be consistent with those of large databases such as Hap-Map to support the generality of the findings. If the haplotypes are not similar to other populations, the findings of studies using those haplotypes may only be significant to those specific subjects.^{38,85}

Many studies have concluded that the tightness of linkage between SNPs is extremely variable within and among loci and populations.²⁶ It has been shown that the ethnic demographics can have profound influence on linkage disequilibrium (LD) between SNPs, haploblock structure, SNP frequency, and estimated effect size drawn from the data.³⁸ Without careful attention to these details, efforts to reduce the number of SNPs required to

characterize genetic variation (using haplotypes, for example)⁵³ might increase the risk of missing valuable information. Reduced cost and resources for genotyping now permit sequencing the entire genomic region of interest, potentially reducing reliance on small sets of SNPs based on haplotypes.

Evaluating the Association Between Genetic Variations and Pain

Candidate Gene Association Study (CGAS)

Candidate gene association study (CGAS) is a powerful means of identifying the common variants influencing complex traits. In a CGAS, the number of statistical comparisons is limited by focusing on polymorphisms in a small set of markers or genes, thus minimizing the required sample size. However, a CGAS relies heavily on choosing genes on the basis of already-revealed biological processes. Due to this limitation, CGAS can only identify genetic risk factors in which the pathophysiology is relatively well understood. The success of a CGAS, ie, finding the responsible genetic variations for a phenotype, is highly dependent on which genes are being investigated. Research findings should provide a rationale for choosing the gene and the particular SNPs as well as provide assurance that bias was controlled in phenotypic or genotypic analysis. Recent development of in silico analyses may enhance the effectiveness of choosing candidate genes and SNPs. The availability of dense murine SNP databases has allowed the development of in silico approaches to gene mapping.^{27,92} Organization of these SNPs into haplotypic blocks has allowed the identification of genes involved in warfarin metabolism,²⁸ opioid-induced hyperalgesia,⁵⁰ tolerance and dependence.⁴⁹ Unfortunately, this technique is currently limited in its power due to the limited number of inbred mouse strains for which the dense SNP information is available.⁹²

It is also critical in conducting and reviewing these studies that the total number of comparisons made in performing the statistical association analysis is appropriately taken into account. Most of the CGASs for pain and responses to analgesics show only weak associations and often fail to replicate in subsequent studies.^{2,14,36,38,71,85,97} Furthermore, annotation of the whole genome is still ongoing. We do not know the functions of all genes and do not even know the exact number of genes. Our currently limited knowledge of pain mechanisms as well as the human genome itself limits our ability to effectively use candidate-gene analysis.

Genome-Wide Association Study (GWAS)

High-throughput genotyping technology allows whole-genome screening in genome-wide association study (GWAS). Several platforms based on chip or bead arrays are available. Fundamentally, these arrays allow the genotyping of more than 1 million SNPs. Thus, a very large percentage of the genetic differences between individual samples are rapidly assessed. The major advantage of this approach is that no specific functional genetic hypotheses are required prior to undertaking analysis. The sample sizes required are, however, increased by the large number of hypotheses that are tested in a GWAS. Results must be corrected for multiple-hypothesis testing. A P value of 5×10^{-8} (equivalent to a P value of .05 after a Bonferroni Correction for 1 million independent tests) is a conservative threshold for declaring a significant association in a GWAS. Because of the very large sample-size requirements, alternative-analysis strategies have been suggested.⁴⁶

Finding the associated SNP is not the same as finding its underlying biology. Candidate SNPs implicated in most GWAS are not on any reasonable list of the “usual suspects”.⁸⁴ Our knowledge about the human genome is not complete yet and many SNPs suggested by GWAS locate in hypothetical genes or intergenic regions. Information for these findings is

not readily available in many studies, though further investigation or annotation can reveal the underlying biology at a later date.

Another reason for this difficulty in connecting GWAS findings to biologic function is the LD of SNP markers. It is easily misunderstood that an associated SNP must have some function. However, the associated SNP may simply be part of a larger block of SNPs in LD which may involve a functionally changed SNP at some distance or even on a separate gene. It is more likely that SNPs on arrays should be considered as location markers, and that more detailed examinations of the region in which the SNP is located are required after the preliminary association is made. Due to the limited number of assayed SNPs or stringency in filtering of data in GWAS, a truly related effector SNP may not be screened while distant SNP markers being in LD show significant associations. Statistically associated SNP markers cannot explain the causative biology in this situation. Further investigation of genetic polymorphisms in a region that is in strong LD with a SNP found by GWAS demonstrates that this multilayer-study design of dense genotyping around the candidate region can reduce the risk of missing important associations caused by extensive filtering of GWAS data. Resequencing the upstream and downstream area of a GWAS finding can reveal novel causative SNPs, which are not annotated in the databases yet, as well as detect missed SNPs.

Multi-Stage Association Studies

A suggested alternative approach is designing a study with multiple stages.^{79,84} First, a relatively small number of samples, at least a few hundred, are used in a preliminary GWAS to identify 1 or 2 candidate genomic regions.⁴⁷ These candidate regions are then genotyped more densely in experiments involving a few hundred SNPs with a larger sample using custom-designed SNP assays. After narrowing the focus to around 10 candidate SNPs, the tested sample can then be enlarged up to a few thousand cases and controls. Individual SNP assays may be appropriate at this level. Success in using this strategy has been reported^{76–78} but collecting several thousand patients diagnosed with a single painful condition to result in a homogenous population is very challenging. Nationwide or worldwide consortia are suggested to enhance the success of GWAS investigating responsible genetic variations for pain.⁸⁷ To enhance the effectiveness of GWAS requiring extensive resources for complex traits, efforts to archive and distribute the results of studies that have investigated the interaction of genotype and phenotype have been established in other research areas already, as in the DataBase of Genotype and Phenotype (db GaP), Genetic Association Information Network (GAIN) or The Cancer Genome Atlas (TCGA). Such studies include GWAS, medical sequencing, and molecular diagnostic assays, as well as associations between genotype and nonclinical traits.

Regardless of the potential power of the multistage association studies in pain research, there are still many barriers. Even with highly heritable phenotypes such as height (heritability estimates ~90%), the most significant SNP (rs1042725 with $P = 4 \times 10^{-8}$) from GWAS with ~5,000 individuals followed by extensive genotyping with ~20,000 subjects can only explain .3% of the individual variation.⁹⁴ It is estimated that the C allele of this particular SNP can increase adult height by .4 cm. This small contribution of a genetic variation for a phenotype stems from the influences of multiple genetic factors as well as environmental factors. The contribution of each gene likely has a subtle effect on multiple mechanisms, making its signal small. Considering the heritability estimates reported in pain along with other influential factors, pain is likely more complex than height. Therefore, the influence of 1 SNP on a pain phenotype may also be extremely small.

Another limitation of genetic-association study is that it cannot identify the underlying mechanism. Identification of causal variations including 3-dimensional structure of genomic

or complementary DNA, and SNPs predicting amino acid change may be the first step for investigating the genetic role in pain sensitivity. It is then desirable to perform functional tests in vivo and in vitro directly measuring the effect of the SNP to confirm the association and to provide a stronger rationale for the relationship to pain sensitivity. Since microarray technology have also been adapted for RNA and proteins, it is now feasible to collect and analyze information from DNA, RNA and protein level across the whole genome at the same time. This leads functional genomics into the integrated genomics, though this concept is presently just starting to emerge.

Future Tools:Whole Genome Sequencing

Currently, whole-genome sequencing is just at the starting line. Whole genome sequencing of 2 individuals (Drs. Venter and Watson) was done in 2007 and 2008 at a cost of millions of dollars. However, this technology will be widely available in the very near future. Only a few thousand SNPs were possible when the first whole genome association study was introduced, however, 1 million SNPs can easily be scanned these days. It is not too early to predict the whole-genome sequencing (not scanning) of an individual person can be affordable in the next few years. The next generation-sequencing technologies are already available for this sequencing, and the 1000 Genomes Project is currently underway.

Statistical Pitfalls in the Analysis of Genetic Association Studies

Considering the current lack of consensus summarized in Tables 1 to 4, statistical approaches vary highly, based on the nature of the outcome measured and reporting for multiple-test corrections. It should be noted that other problems, including sample size determination, applying appropriate statistics, and using chimeric phenotypes, are also important though they are not described in this review.

Population Stratification

Population stratification can produce false associations since the pattern of genetic variations as well as pain ratings may not be uniform between ethnic populations. This is usually overcome by using an ethnically and racially homogenous sample in genetic-association studies.

An example of the potential problems caused by population stratification can be illustrated with our own group's data. Fig 2 shows a significant association between an SNP from *COMT* (rs4646312) and cold withdrawal time (CWT) in mixed-ethnicity samples (68.6% European Americans and 31.4% African Americans) with shorter CWT in T/T homozygotes. When analyzed separately, this significant association did not sustain in both ethnic groups. T/T in African Americans even showed a nonsignificant trend for longer CWT. In this case, the false-positive association was due to population stratification since African Americans provided 9.2% and 19.8% of the C/C and C/T samples, respectively, while 50.2% of the T/T samples were from African Americans. Because European Americans showed longer CWT, the T/T group containing a higher proportion of African American samples resulted in a shorter CWT in this mixed-ethnicity group.³⁹ Thus, in this study, the CWT difference between groups could not be ascribed to *COMT* polymorphisms. Other types of stratification exist as well. Homogeneity of samples with respect to age and gender is also critical because those factors are known to affect pain phenotypes.

The combination of multiple ethnic groups into a single analysis is problematic when conducting association studies. Because we do not know exactly how much the population stratification affects genetic-association studies, statistical corrections of mixed populations may not be entirely effective. Therefore, it is a good general practice to minimize the influence of population stratification by using a single ethnic population whenever possible.

However, we might seek to analyze such mixed populations when these data are available from studies that have already been performed and because clinical populations are of course often mixed. Some researchers have attempted to address this problem by verifying similar genotype frequencies of candidate SNPs between major subgroups, eg, Caucasians, and then combining small remaining subgroups into a single nonmajor subgroup. This approach has been recently debated, the main issue being under what circumstances is combining populations for analysis valid.¹⁶ Not only must investigators rule out stratification between the major subgroup and the nonmajor subgroup but also within any populations combined to form the nonmajor subgroup used in analysis.

Fig 3 illustrates the potential risk of mixing ethnicities in genetic association studies. Initially, it seems to be reasonable to mix and analyze association of an SNP of *COMT* in Caucasians (C/C = 60, C/G = 168, G/G = 92) and nonCaucasians (C/C = 49, C/G = 60, G/G = 36) because their allele frequency is the same as C:G = 45:55 ($\chi^2 = 3.593$, df = 2, $P = .166$). However, there is a problem in that the nonCaucasian group was composed of African Americans (C/C = 49, C/G = 60, G/G = 36; C:G = 40:60), Hispanics (C/C = 11, C/G = 30, G/G = 25; C:G = 33:67), and Asians (C/C = 8, C/G = 48, G/G = 40; C:G = 55:45), ($\chi^2 = 30.053$, df = 6, $P = 3.84 \times 10^{-5}$). Only when combined did these subgroup differences disappear. A putative nonCaucasian grouping should not be treated as a single sample unless it is proven that allele frequencies are the same in African Americans, Hispanics and Asians. A stratified meta-analysis in which each ethnic group is analyzed separately and the summary results combined using meta-analysis can be applied with caution. However, it is still recommended to genotype a number of widely spaced null SNPs (preferably >100; preferably ancestry-informative markers) in addition to the candidate SNPs for combining subgroups without risk of population stratification. PCA with many null markers can provide an effective way to diagnose potential population stratification.³ Various approaches including genomic control, structured association, delta centralization, and Eigen-strat have been suggested at the analysis stage but only for the residual substructure uncontrolled by design or for unidentifiable cryptic stratification.^{13,56,69,96}

Genomic differences between ethnic and racial populations have been characterized to some degree. For example, haplotype blocks in African populations are shorter than those in Caucasians, reflecting the origin of the human race in Africa.²⁶ This means the LD between investigated SNPs is often different between ethnic populations (<http://www.hapmap.org/>).^{32,91} Fig 4 provides a diagram of distinct *GCHI* haploblocks in Caucasians and Africans demonstrating distinct structures. Thus, haplotypic analysis of data from racially mixed populations may be problematic and must take into account race-related differences in the haplotypes used in the analysis. It is well recognized that using small mixed ethnic samples that may result in false associations due to the population stratification.^{9,24,30,39}

Correction for Multiple Comparisons

If one looks at multiple genetic loci and tests them against multiple clinical endpoints, the likelihood of false-positive results goes up multiplicatively. It should be considered that by chance alone, 5% of these tests will meet the conventional α level for significance. If one looks at 46 SNPs and tests them against 3 clinical endpoints³⁶ with conventional α level, the probability of a chance finding is multiplied by 138 times, which results in 6.9 false-positive findings. If the assayed SNPs are increased as in GWAS up to 1 million, the false-positive findings are 50,000 for 1 endpoint.

To overcome false-positive error in genetic-association studies, corrective methods for multiple testing such as Bonferroni corrections have been applied.⁵⁷ However, this highly conservative method of correction increases the risk of false-negative errors. For a GWAS, a Bayesian formula is suggested to attenuate the overly conservative Bonferroni correction.

This method approximates the P -value threshold for GWAS with ~500,000 markers as $2.6 - 4.2 \times 10^{-7}$.^{25,47} A Bayesian formula is applied to obtain >.95 posterior probability of a correct inference of association to a particular gene²⁵ and modified to take into account recent estimates of the total number of genes in the human genome to approximately 20,000, resulting in these modified P -value thresholds. It is particularly applicable to the studies with the assumption that significant markers are not themselves functional variants but rather indicate neighboring genetic loci to be examined in detail.⁴⁷

There are several other alternatives such as the permutation test¹⁰ or false discovery rate analysis⁸³ but it is not yet clear which is the optimal method. Benjamini-Hochberg⁴ proposed a novel approach, the False Discovery Rate (FDR), to account for multiple comparisons. The FDR controls the expected proportion of incorrectly rejected null hypotheses in a list of rejected hypotheses. The Bonferroni correction is very conservative and permutation-based methods such as the re-sampling method of Westfall and Young⁹⁵ are computationally very intensive. The FDR method has been refined to increase its accuracy, applicability, and power.^{82,83} The FDR-adjusted P values can be calculated and reported to determine whether the observed P values are still significant after taking into account multiple comparisons. CGASs as well as GWASs are vulnerable to type I error. If one looks at multiple genetic loci and tests them against multiple clinical endpoints, the false-positive problem goes up multiplicatively. Therefore, the data should be interpreted with a list of all loci ever examined in the data-set of the CGAS.⁵⁷

There are other important issues in genetic analysis which are not discussed in this manuscript. For example, what should be done about rare genetic variations? Including them in analyses can lead to loss of power because there are too many degrees of freedom. How to interpret small marginal association easily denied by multiple-test corrections? A variant with small marginal effect is not necessarily clinically insignificant: It might turn out to have a strong effect in certain genetic or environmental backgrounds, and in any case, might give clues to mechanisms of disease causation.

Recommendations for Clinical Genetic Pain Research

Population stratification, lack of adequate sample size, inappropriate choice of genetic variations, inevitable type 1 and 2 errors, and improper statistical analysis along with publication bias are recognized problems in genetic research.^{3,30} Additionally, the heterogeneity of pain and the definition of pain models also bring complexity to study design and interpretation. The presence of these factors may produce spurious results and misleading conclusions in genetic studies of pain in humans. Only studies with careful designs followed by replication from independent groups and supportive laboratory data can be considered well validated.⁶⁸ It is likely that we will face negative findings much more frequently than positive associations given the obstacles presented above when examining the contributions of individual genes. However, positive findings tend to be known to researchers more often than negative findings, even for the same genetic variations, probably due to the publication bias.

Fig 5 presents a schematic approach to reporting possible sources of error in conducting genetic studies of pain modified from the CONSORT guideline for reporting clinical trials data.⁶³ While new tools like individual whole-genome sequencing, detection of copy-number variation, and runs of homozygosity may potentially add power to study designs, the basic genetic and statistical issues discussed here will continue to apply. Special attention to the following should be considered in evaluating genetic findings in clinical pain research:

Size of sample relative to expected genetic effect

Definition of phenotype and genotype (SNP vs haplotype)

Stratification, HW equilibrium, and other sample population characteristics

Plausibility of the genes under study affecting the phenotype

Orthogonal data supporting hypothesis-like functional data suggesting differences in gene-product amount or function

Replication studies

Statistical issues with multiple comparisons, etc.

Conclusion

Genetics offers the field of pain medicine the opportunity to gain new insights into the pathophysiology of acute and chronic pain as well as differences in individuals' responses to treatments. However, the use of genetic approaches to these questions is highly complex. In general, small studies performed on nonhomogeneous populations with poorly defined endpoints will not provide useful data. Research teams will need to contend with sometimes difficult issues related to enrollment requirements, study designs, and approaches to analysis to avoid the many pitfalls presented in this review. Fortunately, these obstacles are not insurmountable. If we can maintain an open and collaborative approach to performing the relatively large studies required for reliable genetic analysis, this approach may provide new insights into pain mechanisms and new treatments for pain sufferers.

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Figure 1.

Any portion of genomic DNA can influence phenotype by changing promoter activity (A), small RNA interference (B), amino acid change (C) or mRNA stability (D and E).

Figure 2.
False-positive findings caused by population stratification.

Figure 3.

Example of hidden population stratification by comparing Caucasian to all nonCaucasians. The allele frequency for Caucasian and nonCaucasian is the same (45:55). However, the allele frequency varies among the individual ethnic populations in nonCaucasians for African Americans, Hispanics and Asians (unpublished data of *COMT* SNP, rs5746846 by Kim et al).

Figure 4. Different ethnic groups have different haplotype patterns. Confidence-interval method was applied to generate haploblocks. Three blocks (12 kbs with 16 SNPs, 39 kbs with 23 SNPs and 5 kbs with 7 SNPs) are identified in Caucasians while 4 blocks (5 kbs with 7 SNPs, 21 kbs with 25 SNPs, 26 kbs with 16 SNPs and 3 kbs with 7 SNPs) are identified in Africans. Generally, haplotype blocks in African populations are shorter but greater in numbers in a genomic region than those in Caucasians, reflecting the origin of the human race in Africa. **(a)** Haplotype of *GCH1* in Caucasians from HapMap data. **(b)** Haplotype of *GCH1* in Africans from HapMap data.

Figure 5.
CONSORT-like guideline for reporting genetic association data (modified from CONSORT guideline for reporting clinical trial data).

Table 1

Genetic Association Studies in Cancer Pain

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
<i>ABCB1/MDR1</i> 3435C > T	Campa et al Clin Pharmacol Ther (2007)	Caucasians, mixed, 145	Verbal rating pain score and NRS	MANOVA (Bonferroni correction)	Pain relief by morphine therapy is significantly associated with <i>ABCB1/MDR1</i> . Combination of C/C of <i>ABCB1/MDR1</i> and G/G of <i>OPRM1</i> show lower response to morphine.	Using <i>ABCB1/MDR1</i> , patient can be classified into strong responder, responder and nonresponder.
<i>COMT</i> 158	Rakvag et al Pain (2005)	Caucasians, mixed, 207	Morphine consumption	Jonckheere-Terpstra test (not mentioned)	Val allele needs more morphine	Genetic variation in <i>COMT</i> may contribute to variability in the efficacy of morphine in cancer pts
<i>COMT</i> 158	Reyes-Gibby et al Pain (2007)	Caucasians, mixed, 207	Morphine consumption	Multivariable logistic regression analyses (not mentioned)	Val allele needs more morphine. <i>COMT</i> Met and <i>OPRM1</i> A combination requires lowest morphine for achieving pain relief	<i>COMT</i> and <i>OPRM1</i> influence on morphine efficacy either independently or together
15 SNPs from <i>COMT</i>	Ross et al Cancer (2008)	Mixed, mixed, 228 cancer patients	Morphine consumption	Stepwise linear regression analysis (not mentioned)	No association found	Genotype did not correlate with morphine dose. <i>COMT</i> and <i>MDR-1</i> genotypes were correlated with morphine related central side effects but not with pain.
<i>CYP2D6</i> *10 (100C > T inducing <i>Pro34Ser</i>)	Wang et al Eur J Clin Pharmacol (2006)	Chinese, mixed, 63 out of 70	Tramadol consumption (2, 4, 24 and 48 hours), pain VAS	ANOVA, Kruskal-Wallis (not mentioned)	Consumption of tramadol in group without <i>CYP2D6</i> *10 allele is higher than other groups at 4, 24 and 48 hours. No difference in pain intensity.	<i>CYP2D6</i> *10 allele impact on analgesia with tramadol
<i>IL-6</i> -174 G > C, <i>IL-8</i> -251 T > A, <i>TNF-α</i> -308 G > A	Reyes-Gibby et al Cancer Epidemiol Biomarkers (2007)	Caucasians, mixed (446), lung cancer	Pain intensity	Chi square and multivariable logistic regression analysis (no multiple test correction)	<i>IL-8</i> -251 T/T shows lower pain intensity compared to T/A and A/A	Cytokine genes influence on pain in white patients with lung cancer
<i>IL-6</i> -174 G > C, <i>IL-8</i> -251 T > A, <i>TNF-α</i> -308 G > A	Reyes-Gibby et al Cancer Epidemiol Biomarkers (2007)	African Americans, mixed (125)	Pain intensity	Chi square and multivariable logistic regression analysis (no multiple test correction)	No association found	No association found
<i>IL-6</i> -174 G > C, <i>IL-8</i> -251 T > A, <i>TNF-α</i> -308 G > A	Reyes-Gibby et al Cancer Epidemiol Biomarkers (2007)	Hispanics, mixed (35)	Pain intensity	Chi square and multivariable logistic regression analysis (no multiple test correction)	No association found	No association found

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
5 SNPs from MDR1	Ross et al Cancer (2008)	Mixed, mixed, 228 cancer patients	Morphine consumption	Stepwise linear regression analysis (not mentioned)	No association found	Genotype did not correlate with morphine dose. COMT and MDR-1 genotypes were correlated with morphine related central side effects but not with pain.
<i>OPRM1</i> 118 A > G	Campa et al Clin Pharmacol Ther (2007)	Caucasians, mixed, 145	Verbal rating pain score and NRS	MANOVA (Bonferroni correction)	Pain relief by morphine therapy is significantly associated with <i>OPRM1</i> A118 G. Combination with C/C of ABCB1/MDR1 and G/G of <i>OPRM1</i> show lower response to morphine.	Using <i>OPRM1</i> , patient can be classified into strong responder, responder and nonresponder.
<i>OPRM1</i> 118 A > G; -172 G > T; IVS2 + 31, IVS2 + 691	Klepstad et al Acta Anaesthesiol Scand (2004)	Caucasians, mixed, 99 (207)	Morphine consumption and serum concentration	ANOVA (not mentioned)	G/G subjects show higher morphine dose with higher serum concentration	G/G patients need higher morphine doses to achieve pain control.
<i>OPRM1</i> 118 A > G	Reyes-Gibby et al Pain (2007)	Caucasians, mixed, 207	Morphine consumption	Multivariable logistic regression analyses (not mentioned)	G allele needs more morphine; COMT Met and <i>OPRM1</i> A combination requires lowest morphine for achieving pain relief	<i>COMT</i> and <i>OPRM</i> influence on morphine efficacy either independently or together

Table 2

Genetic Association Studies in Chronic Non-Cancer Pain

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
SNPs from ADRA1C (α -adrenergic receptor 1C), ADRA2A (α -adrenergic receptor 2A), and ADRA2C (α -adrenergic receptor 2C)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found	No association with genes encoding α -adrenergic receptors and sciatica
<i>ACE I/D</i>	Huhne et al Eur J Pain (2004)	Germans, mixed, 48 CRPS I and 21 control relatives	Allele and genotype distribution between patient groups and controls	Chi square test (not mentioned)	No association found	<i>ACE gene polymorphism I/D is unlikely a predisposing factor for CRPS I</i>
<i>ACE I/D</i>	Rommel et al Pain (2006)	Europeans, mixed, 23 McArdle's patients and 56 controls	Genotype and allele distribution between patients and controls	MANOVA, chi square and Mann-Whitney U test (not mentioned)	No association found	Gender-related genetic factors as well as maladaptive pain-related coping may contribute to the development of such a chronic pain symptom
<i>ADRB2 haplotype H1, H2, H3</i>	Diatchenko et al Am J Med Genet (2006)	Caucasians, females, 15 chronic orofacial pain patients out of 210	Gene expression, psychological factor, blood pressure, TMD	Relative risk (Bonferroni correction)	Gene expression is lower in H1 and H1 including heterozygotes show low frequency of chronic orofacial pain	Either positive or negative imbalances in ADRB2 function increase the vulnerability to chronic pain conditions such as TMD
<i>AGC1 (aggregran gene) VNTR</i>	Solovieva et al Pain (2007)	Caucasians, males, 132, low back pain	Degenerative change confirmed with MRI	Chi square test (not mentioned)	A26 allele was significantly overrepresented among persons with dark nucleus pulposus	The findings provide additional support for the role of the aggregran gene VNTR in intervertebral disc degeneration
<i>ARA2C Del 322-325, ARB1 389 Gly > Arg, ARB2 16 Arg > Gly, ARB2 27 Gln > Glu</i>	Park et al Am Heart J (2006)	Koreans, mixed, 82 angina and 114 controls	Allele and genotype distribution between patients and controls	Multiple regression logistic analysis (Bonferroni correction)	ARA2C Del 322-325 and ARB2 27 Gln > Glu were associated with angina prevalence	The ARA2C del allele carrier state and ARB2 27 Gln allele homozygote state were identified as novel risk markers of angina in Koreans
3 SNPs from BCL11A	Lettre et al PNAS (2008)	1275 African Americans, 895 African Americans and 350 Brazilians, mixed, sickle cell anemia patients	Pain crisis rate	Poisson regression and ANOVA (not mentioned)	Effect of individual SNPs was not significant. However, markers associated with HbF level in aggregate, have power	Together, HbF-associated SNPs associate with pain crisis rate in SCD patients.

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
SNPs from CHRM1 (cholinergic receptor, muscarinic 1)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found to predict pain crisis in SCD.	No association with CHRM1 and sciatica
1323 SNPs from chromosome 10q22-q23	Anttila et al Am J Human Genet (2008)	Europeans, mixed, 460 migraine and 1215 family members	Linkage analysis and PLINK	Linkage analysis, PLINK (Bonferroni correction)	Highly significant linkage was found with the locus on 10q22-q23. No association found with SNPs	Although no association was found, 4 regions of 10q22- q23 warrant follow-up studies
rs2073711(+1184 T > C), CILP -45C > T, -12 T > C, -19 T > C, +19 G > A, +3496 G > A	Virtanen et al J Med Genet (2007)	Caucasians, mixed, 243 discogenic sciatica and Chinese, mixed, 692 normal	Disc herniation, MRI NDD score	Chi square test (Bonferroni correction)	No association found	<i>CILP is not a major risk factor</i>
SNPs from CNR1 (cannabinoid receptor 1)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found.	No association with CNR1 and sciatica
COMT 158	Armero et al Eur J Pain (2005)	Europeans, mixed, 144 neuropathic pain and 139 controls	Genotype and allele distribution between patients and controls	Chi square and Fisher's exact test (not mentioned)	No association found	Functional Val158Met polymorphism of COMT gene is not associated to increased susceptibility to neuropathic pain
COMT 158	Cevoli et al Eur J Neurol (2006)	Italians, mixed, 103 headache with drug abuse, 101 migraine patients and 117 controls	Allele distribution between patients and controls	Chi square, Markov chain procedure and Fisher exact test (not mentioned)	No association found	COMT 158 is not associated with headache
COMT158	Hagen et al BMC Musculoskelet Disord (2006)	Not clear but mainly Caucasians, mixed, 1529 MSC patients out of 3017	Allele distribution and standardized Nordic questionnaire	ANOVA and chi square test (Bonferroni correction)	Genotypic distribution of 158 are similar between controls and MSCs	No significant association found between COMT158 and MSCs
COMT158	Hagen et al J Headache Pain (2006)	Not clear but mainly Caucasians, mixed, 982 headache out of 2451	Allele distribution between patients and controls	Chi square test (not mentioned)	No significant difference between headache sufferers and normals. Non migrainous headache was found less in val/ val than among other genotypes.	No or weak association between COMT 158 and headaches
COMT RFLP by NdaIII	Park et al J Clin Neurol (2007)	Koreans, female, 97 migraine	Genotype and allele distribution	t-test and chi square test (not mentioned)	No association found. L allele showed	COMT polymorphisms do not

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
<i>COMT</i> rs2097903, rs6269, rs4633, rs4818, rs4680, and rs165599 with haplotype	Vargas-Alarcon et al Arthr Res Ther (2007)	57 fibromyalgia and 33 control Mexicans, 70 FM and 80 Spanish controls	Allele distribution between patients and controls	Chi square, Kruskal-Wallis, Fisher's exact test (not mentioned)	In Spanish patients, there was a significant association between 3 SNPs (rs6269, rs4818, and rs4680) and the presence of FM. In Spanish patients with the 'high pain sensitivity' haplotype (ACCG), the disease, as assessed by the FIQ, was more severe. Mexican patients displayed only a weak association between rs6269 and rs165599, and some FIQ subscales.	Preliminary association was found between COMT genetic variations and FM
<i>CYBA</i> 242 C > T, 640 A > G	Mashiba et al Circ J (2005)	Japanese, mixed, 162 vasospastic angina, 61 microvascular angina, 61 nonresponder angina	Allele and genotype distribution between patient groups	t-test, Fisher's exact probability test, multiple logistic regression analysis (not mentioned)	No association found	No association found
SNPs from DRD2 (dopamine receptor)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found.	No association with DRD2 and sciatica
48 bp tandem repeat in exon III of <i>DRD4</i> (dopamine receptor 4 gene)	Cevoli et al Eur J Neurol (2006)	Italians, mixed, 103 headache with drug abuse, 101 migraine patients and 117 controls	Allele distribution between patients and controls	Chi square, Markov chain procedure and Fisher exact test (not mentioned)	Allele 4 of DRD4 is overrepresented in migraine	Genetic variability of DRD4 is involved in the predisposition to episodic migraine
<i>ENDRA</i> -231 G > A	Lisi et al Headache (2006)	n/a, mixed, 97 headache and 67 controls (child)	Genotype distribution and allele frequencies between patients and controls	Chi square test (not mentioned)	No significant differences found	<i>ENDRA</i> -231 G > A is neither associated with primary juvenile headache nor significantly correlated with main clinical features
<i>eNOS</i> 298 <i>Glu</i> > <i>Asp</i>	Ogimoto et al J Mol Med (2005)	Japanese, mixed, 12 CSA by acetylcholine, 138 nonresponder	Allele and genotype distribution	Chi square test, logistic regression analysis (not mentioned)	The frequency of <i>Asp</i> variant was significantly higher in CSA patients.	<i>Asp</i> 298 variant of <i>eNOS</i> may be associated with CSA in HCM patients

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
frequency of haplotype based on RFLP (-397 T > C and -351A > G) of ESR1	Kang et al Int J Oral Maxillofac Surg (2007)	Koreans, female, 100 TMJ OA with 74 controls	between patient groups Haplotype distribution among mild, moderate and severe pain groups	Chi square test (not mentioned)	No difference between patient and controls. But presence of PX haplotype in patients is significantly associated with pain intensity.	<i>ESR1 polymorphism may be associated with pain susceptibility in female TMJ OA patients.</i>
<i>FVII 353 R > Q</i>	Jeffery et al Int J Cardiol (2005)	Caucasians, mixed, 247 vessel disease angina and 153 no vessel disease angina	Measures of FVII differences between genotype	Cochran-Mantel-Haenszel test, ANOVA (not mentioned)	There was a significant association between all measures of Factor VII and R353Q, with the Q allele associating with lower levels (but pain was not tested)	There appears to be a moderate protective effect of the Q allele against stable angina
SNPs from GABRA2 (GABA-A receptor $\alpha 2$ subunit), GABRB1 (GABA-A receptor $\beta 1$ subunit)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	BPI result is n/a. No association between SNPs and movement score. Haplotype analysis of 1 of the blocks in the GABA-A receptor $\beta 1$ subunit showed that a haplotype of 11% frequency was associated with less limitation of movement.	Current results may suggest that a common haplotype in the GABA-A $\beta 1$ subunit acts like an "endogenous muscle relaxant" in an individual with subacute sciatica.
34 SNPs from GABRB3, GABRA5, GABRG3 (GABA receptor subunits genes)	Oswell et al Am J Med Genet Part B (2008)	Europeans, mixed, 898 migraine patients and 900 controls	Allele distribution between patients and controls	Chi square test (Spectral Decomposition software analysis)	No association found.	No association with GABA receptor genes and migraine
SNPs from GCH1	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found.	No association with GCH1 and sciatica
Pain protective haplotype based on 15 GCH1 SNPs	Tegeder et al Nat Med (2006)	Caucasians, mixed, 168 surgical disectomy plus mixed, female 202 normal healthy plus mixed, mixed, 188 normal	Frequency of leg pain, Z score of thermal pain, Z score of mechanical pain, Z score of ischemic pain	ANOVA (Bonferroni correction)	Pain protective haplotype show lower frequency of leg pain and Z score of mechanical pain	GCH1 haplotype is a marker of pain sensitivity and chronicity.
SNPs from GLRA1 (glycine receptor $\alpha 1$ subunit)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found.	No association with GLRA1 and sciatica

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
5 SNPs from HBC IL-MYB	Lettre et al PNAS (2008)	1275 African Americans, 895 African Americans and 350 Brazilians, mixed, sickle cell anemia patients	Pain crisis rate	Poisson regression and ANOVA (not mentioned)	Effect of individual SNPs was not significant. However, markers associated with HbF level, in aggregate, have power to predict pain crisis in SCD.	Together, HbF-associated SNPs associate with pain crisis rate in SCD patients.
1 SNPs from upstream of HBG2	Lettre et al PNAS (2008)	1275 African Americans, 895 African Americans and 350 Brazilians, mixed, sickle cell anemia patients	Pain crisis rate	Poisson regression and ANOVA (not mentioned)	Effect of individual SNPs was not significant. However, markers associated with HbF level, in aggregate, have power to predict pain crisis in SCD.	Together, HbF-associated SNPs associate with pain crisis rate in SCD patients.
<i>HFE</i> C282Y, H63D	Alizadeh et al Ann Rheum Dis (2007)	Europeans, mixed, 2095 arthralgia, aged 55 years or over	Prevalence of disease in genotypes	ANOVA and chi square test, logistic regression analysis (not mentioned)	Significant higher frequency in H63D homozygotes compared to non-carriers	HFE H63D may explain the prevalence of arthralgia, chondrocalcinosis and arthritis
<i>HFE</i> C282Y	Feeney et al Heart (2004)	English, male, 630 angina patients and 783 controls	Allele and genotype distribution between patient and controls	Chi square test (not mentioned)	No association found	Possession of HFE C282Y is not a risk factor for angina
<i>HLA-A*3303-B*4403-DRB1*1302 haplotype</i>	Sato-Takeda et al Pain (2004)	Japanese, mixed, 52 PHN and 42 no PHN after herpes zoster and 125 controls	Allele and genotype distribution between patient groups and controls	Fisher's exact test (multiplied by the number of alleles at the locus)	The frequency of risk haplotype was higher only in PHN compared to controls	<i>HLA-A*3303-B*4403-DRB1*1302 haplotype plays an important role in the development of PHN but not in the onset of herpes zoster</i>
SNPs from HTR1A (serotonin receptor 1A) and HTR2A (serotonin receptor 2A)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found.	No association with genes encoding serotonin receptors and sciatica
<i>IL-1a</i> 889, <i>IL-1RN</i> 1812, <i>IL-1b</i> 3954	Solovieva et al Pain (2004)	Caucasians, males, 131, low back pain	VAS pain intensity, MRI	Multiple linear regression (not mentioned)	Pain intensity was associated with the simultaneous carriage of the IL-1aT889 and IL-1RNA1812 alleles (OR 3.7, 95% CI 1.2–11.9). Multiple regression analyses allowing for occupation and disc degeneration showed	The results suggest a possible contribution of the IL-1 gene locus polymorphisms to the pathogenesis of LBP. The possibility of chance findings cannot be excluded due to the small sample size.

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
16 SNPs from IL1A, IL1B, IL1RN, TNFA, IL2, IL4, IL6, IL10, IFNG	Noponen-Hietala et al Pain (2005)	Finnish, mixed, 155 sciatica patients and 179 controls	Allele and genotype distribution between patient groups	Chi square test and ANOVA (not mentioned)	that carriage of the IL-1RNA1812 allele was associated with the occurrence of pain, the number of days with pain and days with limitations of daily activities. Carriage of the IL-1b13954 allele was associated with the number of days with pain.	
<i>IL1RN allele a and b</i>	Walker et al Ann Rheum Dis (2006)	Australians, mixed, 313 haemochromatosis patients and 349 controls	Genotype distribution between patients with joint pain and without joint pain, controls with joint pain and without joint pain	Chi square test (not mentioned)	IL6 15 T > A were significantly different between patients and controls. Haplotype of IL6 were also associated with disease.	This supports the role of IL6 genetic variations in discogenic pain.
<i>TNFA</i> -308 G > A, <i>IL-6</i> -174 G > C, <i>IFG</i> 874 G > A, <i>IL-10</i> -1082 G > A, -819 C > T, -592 A > C, <i>TGFB1</i> 10 T > C, 25 G > C	Oen et al Rheumatology (2005)	Caucasians, mixed, 161 juvenile idiopathic arthritis patients	VAS pain intensity	Chi square test, Fisher's exact test (Bonferroni correction)	IL-6 -174 G/G was positively correlated with VAS. TGFB1 25 G/G showed a protective effect against joint space narrowing	IL-6 genotype and TGFB1 genotype may be useful early prognostic indicators
<i>KL</i> -395 G > A	Imamura et al Clinica Chemica Acta (2006)	Japanese, mixed, 77 angina patients and 331 controls	Genotype distribution between patients and controls	Chi square (not mentioned)	No association found	The -395A polymorphism of the human klotho gene is not a genetic risk factor for VSA
<i>LTA</i> 252 A > G	Ryan et al Atherosclerosis (2008)	Europeans, mixed, 851 MI, 309 UA, 354 SA, 105 HYP patients, 697 controls	Allele distribution between patients and controls	Chi square (not mentioned)	No association found.	No association with LTA 252 A > G and angina
<i>MAOA</i> 1460	Cevoli et al Eur J Neurol (2006)	Italians, mixed, 103 headache with drug abuse, 101	Allele distribution between patients and controls	Chi square, Markov chain procedure and Fisher exact test (not mentioned)	No association found	MAOA 1460 is not associated with headache

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
<i>MCP-1</i> 2518	Bucova et al Clin Chim Acta (2008)	migraine patients and 117 controls Slovakian, mixed, 151 angina pectoris patients and 449 health controls	Mutant genotype G/G distribution between patients and controls	Chi square test (not mentioned)	Mutant GG genotype was over presented in angina pectoris patients.	Chronic stable angina pectoris in Slovak patients is linked to MCP-1-2518 (A/G) SNP.
<i>MnSOD</i> 47 Ala/Val	Arisan et al Prostate cancer and prostatic disease (2006)	Turkish, male, 59 chronic pelvic pain syndrome and 36 controls	Allele and genotype distribution between patients and controls	Multivariate logistic regression analysis (not mentioned)	Allele frequency is significantly different between patients and controls	Oxidative disorder-linked medical health problem can be associated with genetic risk factors
<i>NFKBIL1</i> -62 A > T	Ryan et al Atherosclerosis (2008)	Europeans, mixed, 851 MI, 309 UA, 354 SA, 105 HYP patients, 697 controls	Allele distribution between patients and controls	Chi square (not mentioned)	No association found.	No association with NFKBIL1 -62 A > T and angina
<i>OPRM1</i> 118 A > G	Janicki et al Anesth Analg (2006)	mixed, mixed, 121 noncancer chronic patients and 101 abdominal surgery patients	Pain VAS and opioid consumption	Chi square, Fisher's exact test (not mentioned)	No differences in pain score or morphine consumption in acute surgery patients. G allele is lower in chronic pain patients than acute pain patients. In high-quartile, opioid utilization, chronic patients, the A/A required higher opioid dose than G allele.	<i>OPRM1</i> 118 G allele does not appear to affect opioid analgesic use in acute post operative pain. But G allele is less common in chronic pain patients, especially in those requiring higher dose of opioid analgesics.
<i>PLA2G7</i> 994 G > T	Mashiba et al Circ J (2005)	Japanese, mixed, 162 vasospastic angina, 61 microvascular angina, 61 nonresponder angina	Allele and genotype distribution between patient groups	t-test, Fisher's exact probability test, multiple logistic regression analysis (not mentioned)	No association found	No association found
<i>PON1</i> 632 A > G	Mashiba et al Circ J (2005)	Japanese, mixed, 162 vasospastic angina, 61 microvascular angina, 61 nonresponder angina	Allele and genotype distribution between patient groups	t-test, Fisher's exact probability test, multiple logistic regression analysis (not mentioned)	Allele frequency of G was significantly higher in VSA and MVA. His association was strongly influenced by gender in MVA	There was a significant association between PON1 A632 G and MVA as well as VSA
PYGM R49X	Rommel et al Pain (2006)	European, mixed, 23 McArdle's patients and 56 controls	Genotype and allele distribution between patients and controls	MANOVA, chi square and Mann-Whitney U test (not mentioned)	No association found	Gender-related genetic factors as well as maladaptive pain-related coping may

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
40 bp tandem repeat in 3' UTR of <i>SCL6A3</i>	Cevoli et al Eur J Neurol (2006)	Italian, mixed, 103 headache with drug abuse, 101 migraine patients and 117 controls	Allele distribution between patients and controls	Chi square, Markov chain procedure and Fisher exact test (not mentioned)	Allele 10 of <i>SCL6A3</i> is underrepresented in headache with drug abuse compared to migraine	contribute to the development of such a chronic pain symptom Liability of chronic headache with drug abuse may involve genetic variability at the dopamine transported gene in comparison with migraine
<i>5HTTLPR</i> and <i>STin2</i> of <i>SCL6A4</i>	Szilagyi et al Headache (2006)	Caucasians, mixed, 87 migraine and 464 controls (child)	Distribution of genotype between patients and controls	Chi square test (no multiple test correction)	No difference between patient and controls. But <i>STin2</i> 12,12 show overrepresentation in migration with aura.	<i>STin2</i> polymorphism in <i>SCL6A4</i> is associated with migraine with aura.
SNPs from TAC1 (tachykinin precursor-1)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found.	No association with TAC1 and sciatica
SNPs from TH (tyrosine hydroxylase)	Mishra et al Mol Pain (2007)	n/a, mixed, 290 back pain patients	Bodily pain intensity, movement score	Multiple linear regression and step wise regression (Bonferroni correction)	No association found.	No association with TH and sciatica

Table 3

Genetic Association Studies in Surgically Induced Acute Clinical Pain

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
<i>CYP2D6</i> (PM)	Stamer et al Pain (2003)	n/a, mixed, 271 major abdominal surgery patients	Responder, tramadol loading dose, require rescue medication, pain intensity by NRS, patient's satisfaction	t-test, Mann-Whitney U-test, ANOVA (not mentioned)	PM show higher analgesic consumption, more rescue medication. No difference in pain intensity	<i>CYP2D6</i> genotype has an impact on analgesia with tramadol
13 SNPs from COMT	Kim et al Mol Pain (2006)	Caucasian, mixed, 112 for clinical pain caused by oral surgery	Postoperative pain, analgesic onset time, analgesic request time	ANOVA and t-test (Bonferroni correction)	rs740603 in COMT shows weak association with post-operative pain rating. But this association does not sustain after multiple test correction.	No association between monoamine neurotransmitter system and acute clinical pain response.
<i>CYP2D6</i> *M X N	Stamer et al Pain (2003)	German, mixed, 300 major abdominal surgery patients	Postoperative medication of tramadol between PM and EM	t-test, Mann-Whitney U test, chi square test, repeated ANOVA (not mentioned)	PM needed rescue medication compared to EM. PM showed lower response rate to tramadol	<i>CYP2D6</i> genotype has an impact on analgesia with tramadol
<i>CYP2D6</i> *10 allele (118 C > T)	Wang et al Eur J Clin Pharmacol (2006)	Chinese, mixed, 63 out of 70 gastrectomy patients	Tramadol consumption	ANOVA, Kruskal-Wallis H test (not mentioned)	Homozygous patients consumed significantly higher tramadol	<i>CYP2D6</i> *10 allele has significant impact on analgesia with tramadol
9 SNPs from DRD2	Kim et al Mol Pain (2006)	Caucasian, mixed, 112 for clinical pain caused by oral surgery	Postoperative pain, analgesic onset time, analgesic request time	ANOVA and t-test (Bonferroni correction)	No association found	No association between monoamine neurotransmitter system and pain response is replicated.
ecNOS 4a/4b in intron 4, 298 Glu > Asp	Park et al Heart (2004)	Korean, mixed, 164 acute coronary syndrome and 142 controls	Allele distribution between patients and controls	Chi square, linear association analysis and multiple logistic regression analysis (not mentioned)	Genotype and allele frequencies of A:B polymorphism were different between patients and controls.	Non-BB genotype of the ecNOS 4a/4b polymorphism is a protective factor against the development of ACS
38 GCHI SNPs and haplotypes	Kim et al Mol Pain (2007)	Caucasian, mixed, 112 for clinical oral surgery pain out of 367 experimental pain	Pain intensity, analgesic onset time, analgesic request time, VAS to cold and heat stimuli	ANOVA (mentioned but not applied)	No significant association found	Role of GCHI polymorphisms in pain is weak or negligible
IL1B -511 C > T, IL1RA 86 bp VNTR	Bessler et al Neuroscience letters (2006)	Jewish, female, 76 abdominal hysterectomy	Pain VAS and morphine consumption	Chi square, ANOVA, nonparametric Wilcoxon, Kruskal-Wallis test (not mentioned)	IL1RA polymorphism of medium morphine consumption group is different compared to low and high morphine consumption groups	It is suggested that IL1R genetic polymorphism may contribute to the variation in postoperative morphine consumption
8 SNPs from MAOA and 3 SNPs from MAOB	Kim et al Mol Pain (2006)	Caucasian, mixed, 112 for clinical pain caused by oral surgery	Postoperative pain, analgesic onset time, analgesic request time	ANOVA and t-test (Bonferroni correction)	5 SNPs in MAOA shows weak association with post-operative pain rating. But this association does not sustain after multiple test correction.	No association between monoamine neurotransmitter system and acute clinical pain response.

Genotype	Author	Population (Race, Gender, N) and Stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
<i>MAOB intron 13 A > G</i>	Sery et al Neuro Endocrinol Lett (2006)	Caucasians, mixed, 284 tonsillectomy patients	Postoperative pain up to 5 hours	Mann-Whitney U test (not mentioned)	G allele in males referred higher average pain intensity of post-op pain.	Suggest relationship between MAOB polymorphism and post-operative pain intensity in Czech male population.
<i>OPRM1 118 A > G</i>	Chou et al Acta Anesthesiol Scand (2006)	Taiwanese, mixed, 147 knee patients	Pain VAS and morphine consumption	ANOVA and t-test (not mentioned)	Pain intensity is not significant but morphine consumption is higher in G/G alleles	G/G have a poorer response to morphine for post-operative pain.
<i>OPRM1 A118 > G</i>	Chou et al Anesthesiology (2006)	Taiwanese, female, 80 abdominal hysterectomy patients	Pain VAS and morphine consumption	ANOVA (not mentioned)	Pain intensity is not significant but morphine consumption is higher in G/G alleles	<i>OPRM</i> variation may contribute to interindividual differences in post-operative morphine consumption.
<i>OPRM1 A118 > G</i>	Janicki et al Anesth Analg (2006)	Mixed, mixed, 121 noncancer chronic patients and 101 abdominal surgery patients	Pain VAS and opioid consumption	Chi square, Fisher's exact test (not mentioned)	No differences in pain score or morphine consumption in acute surgery patients. G allele is lower in chronic pain patients than acute pain patients. In high-quartile, opioid utilization, chronic patients, the A/A required higher opioid dose than G allele.	<i>OPRM118 G allele does not appear to affect opioid analgesic use in acute post operative pain. But G allele is less common in chronic pain patients, especially in those requiring higher dose of opioid analgesics.</i>
<i>OPRM1 A118 > G</i>	Sia et al Anesthesiol (2008)	Asian, females, 588 cesarean surgery	Pain VAS and opioid consumption	ANOVA (not mentioned)	A/A patients showed lowest pain ratings and morphine consumption.	Genetic variations at position 118 of <i>OPRM1</i> is associated with interindividual differences in pain scores, self-administered morphine and incidence of nausea.
<i>PTGS1 and PTGS2</i>	Lee et al Clin Pharmacol Ther (2006)	Mixed, mixed, 135 oral surgery patients	VAS, mRNA expression	ANOVA (not mentioned)	G/G at -765 of <i>PTGS2</i> show higher expression of <i>PTGS2</i> . G/G show higher response to rofecoxib while G/C and C/C show greater response to ibuprofen.	SNPs at -765 of <i>PTGS2</i> influence on drug response to COX related analgesics.
13 SNPs from <i>SLC6A2</i> (norepinephrine transporter), <i>SLC6A3</i> (dopamine transporter) and <i>SLC6A4</i> (serotonin transporter)	Kim et al Mol Pain (2006)	Caucasian, mixed, 112 for clinical pain caused by oral surgery	Postoperative pain, analgesic onset time, analgesic request time	ANOVA and t-test (Bonferroni correction)	rs40434 in <i>SLC6A2</i> shows association with AOT while rs2066713 in <i>SLC6A4</i> shows association with onset time of postoperative pain. But these do not sustain after multiple correction.	No association between monoamine neurotransmitter system and acute clinical pain response.

Table 4

Genetic Association Studies in Experimentally Induced Pain

Genotype	Author	Population (race, gender, N) and stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
<i>COMT</i> 158	Birklein et al Pain (2008)	Not mentioned but family data, mixed, 50, cold stimuli	Mean pain rating and time of retraction	ANOVA and chi square analysis	No association was found	<i>COMT</i> polymorphisms is not related to cold pain tolerance.
<i>COMT</i> 158 and haplotypes (<i>APS</i> , <i>HPS</i>)	Diatchenko et al Human Mol Genet (2005)	Mixed, female, 15 chronic orofacial pain patients out of 202, heat, cold and pressure stimuli	Z score combined by 16 different pain score, chronic orofacial pain frequency	ANOVA, Z-statistic (not mentioned)	HPS subjects show higher z score of 16 pain scores. <i>COMT</i> 158 is not significant.	3 major haplotypes determine <i>COMT</i> activity in humans that inversely correlates with pain sensitivity and the risk of developing TMD
<i>COMT</i> haplotype (<i>APS</i> , <i>HPS</i> , <i>LPS</i>)	Diatchenko et al Pain (2006)	Mixed, female, 202, pressure, heat, ischemic stimuli	Z score combined by tolerance, threshold together	ANOVA, Z-statistic (Bonferroni correction)	Individuals with HPS/ <i>APS</i> diplotype were the most responsive to thermal stimuli.	The val150 met plays a major role in variation in temporal summation of pain but that other SNPs of the <i>COMT</i> haplotype exert a greater influence on resting nociceptive sensitivity
<i>COMT</i> haplotype (<i>APS</i> , <i>HPS</i> , <i>LPS</i>)	George et al Clin J Pain (2008)	Mixed, mixed, 63, exercise induced delayed onset muscle soreness	VAS pain intensity, evoked pressure pain, muscle torque production and self report disability	Hierarchical regression model, ANOVA, chi square analysis (not mentioned)	Participants with high pain catastrophizing and low <i>COMT</i> activity were more likely to have elevated pain intensity ratings.	The presence of pain catastrophizing and <i>COMT</i> diplotype indicative of low <i>COMT</i> enzyme activity have the potential to increase the risk of developing chronic pain syndrome.
13 SNPs from <i>COMT</i>	Kim et al J Med Genet (2006)	Caucasians, mixed, 367, heat and cold stimuli	VAS and cold withdrawal time	ANOVA (mentioned but not applied)	rs4646312 and rs6269 show associations with cold pain sensitivity in females. No association with heat pain.	Genetic variations in <i>COMT</i> contribute gender specifically to individual variations in cold pain
<i>COMT</i> 158	Zubieta et al Science (2003)	Mixed, mixed, 18 (29), saline injection	Binding potential, McGill pain questionnaire, VAS, infusion rate of saline	ANOVA, Correlations (not mentioned)	Met allele shows low infusion rate to achieve target VAS and less BP to opioid	<i>COMT</i> 158 polymorphism influence the human experience of pain and may underlie interindividual differences in the adaptation and responses to pain
7 SNPs from FAAH	Kim et al J Med Genet (2006)	Caucasians, mixed, 367, heat and cold stimuli	VAS and cold withdrawal time	ANOVA (mentioned but not applied)	rs932816, rs4141964 and rs2295633 show associations with cold pain sensitivity in males. rs4141964 also shows association with cold withdrawal time. No association with heat pain.	Genetic variations in FAAH contribute gender specifically to individual variations in cold pain

Genotype	Author	Population (race, gender, N) and stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
GCH1 haplotype	Tegeder et al Eur J Pain (2008)	Caucasians, mixed, 10 homozygous and 22 noncarrier of haplotype, pressure, thermal and electrical stimuli	VAS and von Frey hair before and after sensitization	Logistic regression and chi square analysis, repeated ANOVA (mentioned but not applied in the result)	Carriers of the particular GCH1 haplotype had higher threshold to mechanical and thermal pain after sensitization	Decreased GCH1 function or inducibility as a result of genetic polymorphism protects against pain
<i>MC1R</i> (R151C, R160 W, D294H)	Mogil et al J Med Genet (2005)	Caucasians, mixed, 47, electrical stimuli	After M6 G: electric intensity at tolerance	ANOVA (not mentioned)	Area under time-effect curve is larger in variant group with bigger effect of analgesia	Non-functional MC1R reduced pain sensitivity and increased analgesic responses to mu opioid drugs.
<i>MC1R</i> (R151C, R160 W, D294H)	Mogil et al Proc Natl Acad Sci (2003)	Caucasians, mixed, 42, ischemic and thermal stimuli	After pentazocine: time of first pain (threshold), time at request stop (tolerance), numerical verbal descriptor of pain intensity and unpleasantness	ANOVA (not mentioned)	Ischemic pain tolerance and rating in women show greater effect with 2 variants	It demonstrates unexpected role of MC1R in k opioid analgesia.
11 SNPs from OPRD1	Kim et al J Med Genet (2006)	Caucasians, mixed, 367, heat and cold stimuli	VAS and cold withdrawal time	ANOVA (mentioned but not applied)	No associations with heat and cold pain responses	Genetic variations in OPRD1 do not contribute to individual variations in heat and cold pain
<i>OPRM1</i> 118 A > G	Fillingim et al J Pain (2005)	Mixed, mixed, 167, pressure, thermal and ischemic stimuli	PPT, thermal pain threshold and tolerance, temporal summation of thermal pain, tourniquet test	ANOVA and chi square test (not mentioned)	G/G subjects show higher pressure pain threshold. G/G show lower heat pain rating in men but higher in women.	SNP of OPRM1 is associated with mechanical pain and this may also be associated with heat pain in a sex-dependent manner.
<i>OPRM1</i> 118 A > G	Loisch et al Behav Neurosci (2006)	Caucasians, mixed, 45, carbon dioxide on nasal mucosa	Pain related cortical potential (carbon dioxide on nasal mucosa)	t-test (not mentioned)	A/G and G/G people show ERP as half as high compared to A/A subjects.	Independently verify moderate pain protective role of the G variant.
<i>OPRM1</i> A118 > G	Oertel et al Pharmacogenet Genomics (2006)	Caucasians, mixed, 20, electrical and chemical stimuli	Percent change of pain response after medication (electrical and chemical)	Power model	G allele show decreased analgesia for electrical stimuli while only homozygous G/G show decreased analgesia for chemical pain.	G/G require more alfetamil than others.
<i>OPRM1</i> 118 A > G	Romberg et al Anesthesiology (2005)	n/a, mixed, 16, electrical stimuli	Pain tolerance (electrical)	NONMEM model	Analgesic response is small and inconsistent with G/A allele compared to A/A.	G allele reduced analgesic response
<i>OPRM1</i> 118 A > G	Zhang et al J Biol Chem (2005)	n/a, mixed, 87 postmortem brain tissue	Peak area ratio of A/G in genomic DNA and cDNA from 8 heterozygotes	A/G ratio in cDNA	A118 mRNA expression is 1.5–2.5 fold abundant than G118 allele.	G allele is a functional variant with deleterious effect on mRNA.
8 SNPs from TRPA1, 12 SNPs from TRPM8, 13 SNPs from TRPV1	Kim et al J Med Genet (2006)	Caucasians, mixed, 367, heat and cold stimuli	VAS and cold withdrawal time	ANOVA (mentioned but not applied)	rs1198795 in TRPA1 shows association with cold withdrawal time. No association with heat pain.	Genetic variations in TRPA1 contribute gender specifically to individual variations in cold pain. But TRPM8 and TRPV1 SNPs

Genotype	Author	Population (race, gender, N) and stimuli	Phenotype	Statistics Used (Multiple Correction)	Results	Conclusion
						are not associated with heat and cold pain.