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Phenotyping and Genotyping Neuropathic Pain

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Abstract Despite ongoing efforts, neither effective treatments nor mechanistic understanding of the pathogenesis of human neuropathic pain exists. Genetic association studies may point to the novel molecules that mediate neuropathic pain, facilitating its understanding and management. Several studies used a candidate gene approach to elucidate genetic contribution to neuropathic pain phenotypes; however, the data is limited and inconsistent. Possible reasons include: sample heterogeneity, underpowered study design, population admixture, poor phenotyping, genotyping errors, and statistical analytical mistakes. This article summarizes and discusses current strategies to optimize population-based association studies of human neuropathic pain focusing on principles of measuring neuropathic pain phenotypes and genotyping techniques. We also consider advantages and challenges of study designs and statistical analyses.

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Introduction

According to the International Association for the Study of Pain (IASP), neuropathic pain is "pain initiated or caused by a primary lesion or dysfunction or transitory perturbation in the peripheral or central nervous system" [1]. It is often referred to as non-nociceptive pain or "deafferentation" pain, which suggests abnormal production of impulses by neural tissue that is separated from afferent input [2]. Many common diseases, such as cancer, stroke, multiple sclerosis, syringomyelia, diabetes, and herpes zoster may produce neuropathic pain. The clinical picture of neuropathic pain is similar in many cases, and clinical features include ongoing spontaneous or evoked pain in an area with sensory loss, positive sensory symptoms such as allodynia and hyperalgesia, wind-up pain following repetitive stimulation, referred pain, and abnormal sympathetic activity [3–5•]. Neuropathic pain greatly impacts the quality of life and is a troublesome situation both for the patient and the physician [6, 7].

Today, pain research is focusing on new molecular methods such as gene therapy, stem cell therapy, genetics, and genomic studies. These methods could provide a new therapeutic approach to neuropathic pain relief [8•]. Recent studies have established that both clinical and experimental pain perception are influenced by genetic factors [9–12•]. Although the relative importance of genetic versus environmental factors in human pain perception remains uncertain, familial clustering and human twin studies clearly demonstrate the importance of genetic factors for variability in human pain perception [13]. Because major

injuries or disease exposures rarely occur together in sibling pairs, association studies in unrelated individuals are the standard method to identify genetic determinants of neuropathic pain.

Association studies, in which the frequencies of common allelic variants are compared in cases and controls, or within cases with different degrees of phenotype expression (cohort studies), have greater power than family linkage studies to detect genetic effects of slight to modest size [14, 15] and have already shown promise [11]. Their main disadvantage, however, is a low replication rate that may be caused by limited sample size, sample heterogeneity, population admixture, poor phenotyping, genotyping errors, or statistical analytical mistakes. Therefore, setting up guidelines for the study design, phenotyping, and genotyping may optimize data collection and analysis and lead to more reliable and replicable data. The purpose of this article is to provide the readers with a strategy to carry out genetic association studies in neuropathic pain patients and to suggest a set of validated tools for neuropathic pain phenotyping. This article also discusses methodologic challenges of candidate gene and genome-wide association studies, as well as several approaches to follow-up the association signal.

Designing Genetic Association Studies

In genetic association studies, population subdivision, recent admixture, and sampling variance can lead to spurious associations between a phenotype and a marker locus or may mask true associations [16]. Because several environmental and socio-demographic factors may interact with genetic polymorphism and influence pain perception, detailed information about the individual should be collected, including age, sex, marital status, occupation, education, religion, employment status, marital status, type of insurance, disability claims, retirement claims, and sick leave. Another factor that potentially contributes to sample heterogeneity is hair color. Recent studies have demonstrated that people with naturally red hair are resistant to subcutaneous local anesthetics, are more sensitive to thermal pain, and prone to prolonged and neuropathic pain [17]. It was also shown that the link between red hair color and pain is genetically determined; [18] therefore, hair color is an important variable in human pain genetic studies.

Another source of population substructure is admixture based on race and ethnicity. Population stratification can occur if cases and controls have different frequencies of ethnic groups, different fractions of ancestry, and when phenotypes of interest, such as neuropathic pain, differ between ethnic groups [16]. The genotyping approach for the detection and correction of population admixture is discussed in the following text.

Clinical homogeneity can be achieved by careful collection of clinical data related to the primary disease, treatments, complications and adverse effects, pathologic laboratory findings, physical examination, detailed information on symptoms, medical history (including any previous pain or pain from independent origin), and family history. All of these data are necessary for patient clustering and appropriate phenotype–genotype analysis.

Data management and quality control, including automatic data entrance with scannable or computer-based forms, tracing system for missing data, electronic records and secure databases, data integrity control, verification, and evaluation, may prevent "chance" findings and misinterpretations and lead to more reliable and replicable association data.

A prospective longitudinal design of pain genetic study is preferable to a cross-sectional or retrospective design and allows the identification of causative relationships between co-morbid traits and other variables and prevents memory bias in pain assessment.

Phenotyping of Neuropathic Pain

A systematic approach of rigorous phenotyping of human neuropathic pain is based on a multidisciplinary characterization of all pain components, including sensory, psychosocial, psychophysical, emotional, cognitive, cultural, and behavioral aspects, as well as the pain's influence on level of functioning and quality of life. Clinical history and clinical examination provide key information on diagnostic features of neuropathic pain, such as sensory deficit, functional abnormalities, spontaneous ongoing and evoked pain, and strange sensations [19••]. The patient history should also clarify pain quality (e.g., burning, lancination, shooting), time course (frequency and duration), location and intensity of pain symptoms, as well as treatments and co-morbidities. The intensity of pain can be assessed descriptively (e.g., "mild," "moderate," "severe," "excruciating"), numerically (e.g., on a scale from 0–10), or using the Visual Analog Scale. If the pain has more than one component (e.g., continuous ongoing pain and superimposed lancinating pain), the intensity of each component should be assessed separately [20•]. The form of the question, the scale used to answer the question, the type (single vs. multiple question) of questionnaire, and the time period over which pain is assessed should be carefully considered. Full phenotypic assessment may be done only in the clinic during in-patient visit, but some parts may also be done via a phone interview or a telephone-based Interactive Voice Response system [21].

Symptom Assessment

Several questionnaires are widely used to assess neuropathic pain, and these tools were validated for different neuropathic conditions. The Neuropathic Pain Scale (NPS) and Short Form McGill Pain Questionnaire (SFMPQ) have received validation in peripheral neuropathic pain conditions and also in central neuropathic pain associated with multiple sclerosis [22]. The painDETECT tool was originally developed to detect neuropathic disease components in patients with chronic low back pain, but it is also useful for identifying other types of neuropathic pain [23]. The Brief Pain Inventory (BPI) has been demonstrated as promising instrument in the evaluation of painful diabetic peripheral neuropathy [24]. Verbal screening tools include the Leeds Assessment of Neuropathic Symptoms and Signs pain scale (LANSS) [25, 26], 12 item Neuropathic Pain Questionnaire (NPQ) [27], a clinician-administered neuropathic pain diagnostic questionnaire (DN4) [28, 29], and a six-item questionnaire known as ID Pain [30]. Each of these screening tools uses between four and nine pain descriptors, three of which (tingling, shooting, and burning pain sensations) are included in all of the questionnaires. The sensitivity (66%–91%) and specificity (74%–94%) of these instruments fall within reasonable ranges [31].

The National Institutes of Health Patient-Reported Outcomes Measurement Information System (PROMIS) Roadmap initiative (http://www.nihpromis.org) has developed, validated, and standardized item banks to measure patient-reported outcomes relevant across common medical conditions, including Pain Impact and Pain Behavior and co-morbid disorders (anxiety, depression, sleep, fatigue). An Internet-based survey was designed and completed by 15,528 controls and 967 chronic pain patients [32]. Pain behavior scores were strongly related to pain intensity and moderately related to self-reported general health status. Additional validation of these tools in neuropathic pain populations is required.

Sometimes, existing standard questionnaires do not provide detailed information on pain localization or sensory disturbances for certain neuropathic pain disorders such as post-mastectomy pain. Newly designed tools should be based on questions or topics identified in the literature and tested in target population [33].

Finally, a novel method of identifying pain subtypes, Standardized Evaluation of Pain (StEP), was validated for neuropathic pain patients. StEP consists of a qualitative yet standardized bedside assessment of pain-related symptoms and signs, and allows distinguishing neuropathic from non-neuropathic pain with high sensitivity and specificity as well as characterizing neuropathic pain sub-phenotypes [34•].

Psychosocial Assessment

Multiple psychological factors have been implicated as potential risk factors for the development of neuropathic pain, including depression [35], anxiety [36], worker's compensation [37], sleep patterns [38], catastrophizing, and somatization [39]. The psychological assessment of neuropathic pain patients ideally includes at least an assessment of mood, physical dysfunction, pain-coping strategies, and social support [40].

Although many measures of mood are available, the Beck Depression Inventory [41] and the Center for Epidemiological Studies–Depression Scale [42] are two of the most frequently used measures of depressive symptoms in the chronic pain literature. Measures of both general anxiety and pain-specific anxiety include the State-Trait Anxiety Inventory [43], the Pain Anxiety Symptoms Scale [44], and the Symptom Checklist 90-Revised [45]. The Brief Symptom Inventory is a well-established screening tool of psychological distress that has been used extensively in chronic pain patients.

Because neuropathic pain is associated with interference in many daily activities, such as work, recreation, socializing, and sleep, several measures of disability or interference in daily activities due to pain may be used for measuring phenotypes, the most common being the Pain Disability Index [46] and the Multidimensional Pain Inventory [47]. A Short Form SF-36 is a generic instrument that measures the mental and physical dimensions of health-related quality of life [48] and has some validation in neuropathic pain conditions.

An emerging body of research indicates a relationship between coping and adjustment in chronic pain patients, especially catastrophizing and seeking social support [49, 50]. The Coping Strategies Questionnaire [51] and the Chronic Pain Coping Inventory [52] are two measures developed specifically for the study of pain-coping strategies.

Finally, a growing body of evidence demonstrates a correlation between neuropathic pain and somatization [53]. The Kohn Reactivity Scale assesses an individual's level of reactivity or nervous system arousability, and the Pennebaker Inventory for Limbic Languidness assesses the frequency of occurrence of 54 common physical symptoms and sensations.

Psychophysical Assessment

Quantitative sensory testing (QST) is a valuable phenotyping tool and is widely used in pain research to detect perception threshold values regarding touch (A beta fibers), warmth (C fibers), cold (A delta fibers), and heat pain (C fibers). QST is applied according to standardized

protocols in order to assess the function of the somatosensory system, and it permits the creation of distinct patient groups according to sensory profiles [54]. The assessment of psychophysical responses includes 1) responses to innocuous stimuli; 2) thermal pain threshold, which reflects cutaneous sensitivity; 3) pressure pain threshold, which reflects the sensitivity of muscle tissue; 4) cold pain tolerance; 5) ratings of the intensity and unpleasantness of suprathreshold thermal stimuli; 6) temporal summation of pain, a measure of central sensitizability; and 7) diffuse noxious inhibitory controls (DNIC), a measure of endogenous pain inhibition that refers to the phenomenon of one noxious stimulus inhibiting the pain of a second noxious stimulus. A full testing session takes about 1 h. Descriptions of the testing methods appear elsewhere. All of these procedures are non-invasive, non-damaging, and have been used in studies of neuropathic pain patients [55].

Phenotypes collected by sensory stimulation include: cold and warm detection thresholds; cold and hot pain thresholds (usually measured by the Thermal Sensory Analyzer; Medoc Ltd., Ramat Yishai, Israel); mechanical touch detection thresholds, pinprick sensitivity, and dynamic mechanical allodynia (assessed with Von Frey filaments); vibration detection thresholds (measured with Rydel-Seiffer tuning fork); and mechanical pain thresholds and pressure pain tolerance (measured with an algometer). Cutaneous heat stimulation is delivered by a diode laser to access the heat hyperalgesia levels, and the tourniquet procedure is used for ischemic pain intolerance assessment; these two procedures, as well as electrical pain, have somewhat limited practice because they are quite lengthy or considered an unnatural stimulus.

In addition to method of threshold detection, a QST method of supra-threshold rating was published recently [56••, 57••] This approach captures not only positive but also negative sensory phenomena and provides quantitative data plus mapping of those findings, all of which would be used for phenotyping.

Autonomic Assessment

Studies have shown that the enhanced sensitivity to painful stimuli in chronic pain patients results from an impairment in central pain regulatory systems that are influenced by resting arterial blood pressure. Many central nervous system pathways that regulate cardiovascular function are also involved in pain regulation. In general, higher levels of resting arterial blood pressure are associated with diminished sensitivity to thermal, mechanical, and ischemic stimuli [58, 59]. Moreover, data suggest that low resting arterial blood pressure is associated with an enhanced state of pain amplification and contributes to the development and maintenance of persistent pain [60].Therefore, resting blood pressure and heart rate may become significant intermediate phenotypes in genetic studies of human neuropathic pain.

Genotyping Neuropathic Pain

Neuropathic pain, as well as other forms of human pain, is clearly a genetically heterogeneous condition. There are many challenges in carrying out a valid genetic study of complex traits [61]. Thornton-Wells et al. have reviewed seven biological phenomena that significantly contribute to the complexity of the genotype–phenotype mapping relationship in common diseases and complex traits that can be broken down into heterogeneity-related and interaction-related factors. Heterogeneity-related factors include allelic heterogeneity, locus heterogeneity, phenocopy, trait heterogeneity, and phenotypic heterogeneity. Interaction-related factors include epistasis or gene–gene interaction and plastic reaction norms or gene–environment interaction [62].

The most efficient approach that can be used to identify genetic markers for human pain is to analyze the additive effects of polygenic variants of multiple functionally related groups of candidate genes. These genes contribute to the structure and/or functioning of pathways in the brain and body, which evolved to respond to danger signals from the internal and external environment, including environmental threat (i.e., stress), tissue damage (i.e., pain), or infection (i.e., inflammation) [63•]. On the other hand, the "risk" genes do not cause complex conditions such as neuropathic pain, but rather predispose the brain and body to physiologically respond to internal and external environmental conditions in ways that lead to symptom production.

Due to the complexity and interdependency of pain pathways, contributing factors, and relevant gene/environment interactions, it is not surprising that genetic studies of pain are still in their infancy. Nonetheless, a number of genes have emerged as potential risk factors for impaired pain processing, including genes coding for opioid receptors, transient receptor potential cation channels (TRPV1), fatty acid amino hydrolase (FAAH), and GTP cyclohydrolase 1 (GCH1). Association studies in pain conditions include several studies in migraine and scattered reports in back pain, postherpetic neuralgia, temporomandibular disorder, fibromyalgia, complex regional pain syndrome, dysmenorrheal, and prostatic pain [12••, 64]. For a few pain candidate genes, there has been limited replication, including *IL6*, *COMT*, and *GCH1*.

There are several approaches to identifying pain-related genes. One approach is to first define a phenotype and then look at gene expression in carriers and non-carriers. Another possibility is to first define a list of candidate

genes that encode key molecules based on their involvement in pain-related pathways (e.g., neurotransmitters, inflammatory mediators, ion channels) and test them in experimental models and in the clinic. Recent studies have tested the significance of individual genes or their combinations in certain pain phenotypes, using functional and/or informative markers. Low back pain and sciatica have been associated with polymorphisms in the interleukin-1 family of genes [65] and *IL-6* [66]. The persistence of herpes zoster pain has been associated with *HLA* polymorphisms [67], and the analgesic response to anti-inflammatory drugs has been associated with prostaglandin gene polymorphisms [68]. Two studies have reported that acute and chronic temporomandibular muscle pain is associated with polymorphisms of *COMT* [11, 58]; however this gene has not been associated with increased susceptibility to neuropathic pain in a Spanish population [69]. Mutations in the genes for neurotrophic factors and their receptors have been shown to be associated with neuropathic pain levels in human sensory neuropathies [70]. Recently, we demonstrated novel genetic associations, including a common missense mutation in *KCNS1* (encoding potassium voltage-gated channel subfamily S member 1) and a common variant in *SCN9A* (encoding for voltage-gated sodium channel subunit alpha Nav1.7) associated with sciatica and phantom limb pain (Belfer et al., unpublished data).

In 2004, we described a method for prioritizing candidate genes and polymorphisms for chronic pain studies by rating each polymorphism in a candidate gene according to the strength of evidence supporting involvement of the gene in pain processing, frequency of the specific variant, and likelihood that the polymorphism alters function [71]. Approximately 250 molecules are involved in pain processing, and many of the underlying genes have common single nucleotide polymorphisms (SNPs). Other types of genetic polymorphisms, including dinucleotide or trinucleotide repeat motifs of variable length, and insertion or deletion of larger portions of DNA, may be also present in coding and non-coding regions, potentially altering protein function or abundance of key regulatory molecules, and thereby influencing the efficacy and toxicity of therapy or resulting in predisposition to disease. However, high-throughput methods for the detection of these polymorphisms are currently unavailable and they may be tested only in single gene genotyping. SNPs are the markers of choice due to their abundance across the human genome, easy identification with current multiplexing technologies, and good potential for functional consequences. These functional consequences may include amino acid change, change in the amount of message or protein expression or function, or association with a clinical phenotype. In case of a lack of putative functional variants in the candidate gene, one can undertake a haplotype-based approach by identifying haplotype block structure in different human populations [72]. Haplotype blocks are combinations of common alleles that occur together over 10-kb to 100-kb lengths of DNA. Over each of these DNA segments, approximately 90% of individuals have one of the two to five most common haplotypes. When loci are present in haplotype blocks, their information can be combined and haplotype can be used as genotype. If approximately six loci are tested per block, one can accurately predict all common variants in that haplotype block, including untested variants that may be the true disease-risk alleles [73]. A panel of these tag SNPs maximizes haplotype information content in the gene and may be used to investigate haplotype associations with pain phenotype.

Based on this method, and using functional and informative (tagging) SNPs, a comprehensive panel of pain candidate genes—the Pain Research Panel—has been created (Beckman Coulter Genomics, formerly Cogenics, Beverly MA). A recently updated version of this panel contains over 3,000 informative SNPs in 350 genes with protein products linked to biological pathways that influence pain sensitivity and/or psychological state. The panel has three major gene clusters with genes influencing peripheral or central pain processing (e.g., voltage-sensitive sodium channels, purinergic receptors, opioid receptors); genes sensitizing sensory pathways or stress sensitivity, mostly through inflammation or tissue injury responses (e.g., neurotrophic factors, bradykinin, interleukins); and genes related to affect, an important dimension of the pain experience (e.g., serotonin receptors 1 and 2, corticotropin-releasing hormone receptors). Many of the genes in these three clusters also produce proteins that mediate the therapeutic effects of pharmacologic agents used to treat pain, inflammation, affect, and mood. At least 200 Ancestry Informative Markers (AIMs) (SNPs that are unlinked to the candidate loci) are also included in Pain Research Panel. AIMs are spread throughout the genome with a distance of more than 100 kb from each other and have extremely different frequencies in different ethnicities. AIMs are used in statistical programs, principally Structured Association and Genomic Control, for the detection and correction of population stratification, estimation of the ancestry of individuals within a sample, and testing for and adjusting the ethnic matching of cases and controls [74].

The Pain Research Panel has already been genotyped in five large independent cohorts with human chronic pain phenotypes, including fibromyalgia and temporomandibular disease [75]. Despite clear advantages of the pain panel genotyping compared with single gene genotyping, there are some disadvantages, including relatively large sample size required and, like any other candidate gene approach, selection bias (because the investigator has to rely upon prior evidence on genes and variants that is limited and/or

controversial). Large-scale studies in homogeneous groups of neuropathic pain patients using a genome-wide screen (i.e., a genome-wide association study [GWAS]) may overcome the selection bias limitation and tremendously facilitate neuropathic pain research in several ways, including identifying entirely novel mediators of disease; focusing ongoing programs of drug development in pre-clinical models on specific pathways or mediators of human neuropathic pain; extracting up to 50% of the variance from studies of non-genetic factors that affect neuropathic pain; providing a better understanding of pain risks, mechanisms, and treatments of individual patients (thus leading to "personalized pain medicine"); and, finally, improving classification of pain disorders [76••].

No one can predict which goals and potential advantages of GWAS might be eventually realized, as the patterns of findings from the first 100 or so GWAS of common human diseases depended on each disease's "genetic architecture" (i.e., how the total heritability of the disease is divided among the common variants in the genome). For some genetic variants with relatively large effects on risk, genetic tests may help predict risk or identify important differences of disease biology in the individual patient. If a polymorphic gene explains only a small part of the population risk for the disease, the discovery of such a variant with small but definite effect on a medical phenotype may, however, prove a principle that will allow for development of a much stronger treatment. Finally, clinical drug trials show that standard pain measures are quite sensitive to modest biochemical effects, that pain is mediated by a well-characterized set of peripheral nerve and spinal cord systems, and that pains caused by a uniform peripheral injury therefore have very similar environmental influences. Thus, neuropathic pain phenotypes may be successfully used in GWA studies and avoid many of the liabilities found, for example, in phenotypes of psychiatric disorders.

Current GWAS technologies, including the Illumina (San Diego, CA) and Affymetrix (Santa Clara, CA) platforms, allow not only genotyping more than 550,000 tag SNPs from HapMap data, but also 100,000 additional markers that specifically target regions of common copy number variation, providing data on up to 1.2 million SNPs and 11,000 copy number variants. We believe the powerful new methods of unbiased interrogation of the whole genome based on systematically grouped well-phenotyped patients possess enormous potential for progress in treating and understanding neuropathic pain.

Follow-Up Studies

After identification of the association between the genetic locus and the phenotype, the next step is to replicate and validate the findings. This may include genotyping of the novel susceptibility loci in the same data set using an alternative genotyping platform, genotyping of the "hits" in independent data sets with the same or similar phenotypes, and extensively analyzing functional mechanisms responsible for the association signals. Current bioinformatics tools provide information on possible function, based on the location of the "hit" on the gene sequence. Accordingly, one can measure differential allelic expression, total amount of RNA, related protein, or gene product in homozygotes for each allele and heterozygotes. If it is unlikely that the SNP has a causative allele, and rather reflects the signal from another one (acting as proxy, or sentinel, for the true disease variant (s)), next-generation sequencing may reveal rare and hidden mutations that might be true susceptibility loci. Further replication and functional validation will be required. Strategies on comprehensive follow-up and uncovering the mechanisms of genetic contribution to the phenotype are discussed in the recent review by Donnelly [77•].

Statistical Considerations

Single Marker and Haplotype Analysis

The initial round of association analyses in candidate gene or GWAS comprises univariate testing of single SNPs (i.e., single marker analyses) using t tests, contingency tables, analysis of variance, or regression analysis. More biologically relevant, the data may also be analyzed assuming a pre-specified genetic penetrance model, such as an additive genetic model (i.e., the combined effect of two genetic alleles are equal to the sum of their individual effects to cause the affection), dominant model (i.e., one allele is sufficient to cause the affection), recessive model (i.e., two disease-causing alleles are required to cause the affection), and multiplicative model (i.e., the joint effect of two genetic alleles is the product of their individual effects). Haplotype analysis of multiple SNPs in a chromosomal region of interest provides an in-depth analytical approach that is not only biologically relevant but also is more powerful than the analysis of single markers, especially when a disease-causing variant is not typed or when there are multiple causative alleles [78]. Haplotypes are mostly estimated using Clark's algorithm, expectation-maximization (EM) algorithm, Bayesian algorithm, and their various derivatives. Because both the Clark's algorithm and EM algorithm are sensitive to deviations from the Hardy-Weinberg equilibrium (HWE), an equivalence test for HWE is strongly recommended. Bayesian algorithms, however, have no such assumptions and should be used when HWE does not hold for certain markers. The software SAS/Genetics (SAS version 9.2; Cary, NC) currently offers easy

procedures to the estimation of haplotype frequencies using both EM and Bayesian algorithms.

Several methods are available for testing associations with haplotypes. The widely used method is to group individuals with the sample type of haplotypes and then to do a simple pair-wise comparison of different groups. However, this simple method does not take the haplotype uncertainty into account. Because haplotypes are not observed but estimated, this method might only work when haplotypes could be estimated with high certainty (e.g., the loci spanning the haplotypes are in high linkage disequilibrium [LD]) and will result in a decrease of the sample size and power of the study. Other options of haplotype analysis include likelihood-ratio or a score test. In addition, Zaykin et al. [79] proposed a haplotype trend regression that has the option of including covariates and interactions into modeling. At the whole-genome level, a variable-sized sliding-window framework has been proposed [80].

Multivariate Association Analysis

Most pain-related phenotypic measurements are intricately linked and intertwined. Given the fact that neuropathic pain phenotype can only be represented or measured by integrating multiple correlated "intermediate" phenotypes, the correlation structure of these "intermediate" phenotypes should be modeled, and multivariate analysis will be more powerful.

Multiple Testing Issues

Failure to appropriately adjust for multiple testing may produce excessive false positives (e.g., Type I error). The Bonferroni method, the permutation-based method, and the false discovery rate (FDR) method are the approaches most often applied to control for this error. The Bonferroni method, assuming independence of each test, is easy to compute but is too conservative in the presence of LD among SNPs. A slightly modified step-wise approach orders the *P* values from smallest to largest, uses a different cut-off for each *P* value, and is less conservative. The permutation-based method can account for the LD among SNPs but is most computationally intensive in large-scale association studies. The FDR is the expected proportion of erroneous rejections (i.e., false positives) among all rejections, and works well even when applied to markers in LD. Prioritized subset analysis (PSA), or a two-tier approach, can be used to evaluate candidate genes. In this approach, SNPs are first divided into high (e.g., first tier) and low (e.g., second tier) priority tiers based on prior evidence of candidate genes or linked regions or clinical criteria. Finally, a newly proposed weighted hypothesis testing [81], in which the association *P* value is weighted according to genome annotation information or results from prior association studies, can optimize the average power of the genetic association study without affecting control of Type I error.

Sample Size

Two factors affect sample size: 1) the frequency of the SNPs and 2) the size of the effect one wishes to detect. Because the average frequency of SNPs is 10% to 15%, larger cohorts are required. An allele that increases the risk of severe pain by 80% (i.e., relative risk = 1.8) can be detected in a study of fewer than 1,000 cases and controls. Unfortunately, only a minority of replicated SNPs from GWAS have had this large an effect. More commonly, relative risk values have been 1.2 or less, and therefore only detected with studies of 10,000 or more individuals. However, relative risk values may be dramatically enhanced by thoughtful choice of the phenotype to maximize homogeneity of the population and the impact of environmental factors on the phenotype. Detailed considerations on sample size are summarized in our previous article [71].

Meta-analysis in Association Studies

Increasing sample size by combining data from multiple studies, meta-analysis provides a methodologic tool to increase the power to detect genotype-phenotype associations and to investigate the heterogeneity of these associations across different datasets and study populations. Types of meta-analysis usually include either pooling *P* values or pooling estimates of effect size of different studies. Considerable expertise and strict adherence to certain methodologic steps are indispensible in the application and interpretation of meta-analysis methods [82].

Conclusions

Research in human genetic polymorphism is expected to help expedite the realization of genomic personalized medicine (i.e., the selection and dosage of medication determined according to each individual patient's specific set of genetic and non-genetic factors). Applying new phenotyping and genotyping approaches to the association studies of neuropathic pain will improve data quality and facilitate fundamental investigations of this pain on cellular and molecular levels, leading to better therapeutic management.

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- • Of importance
- •• Of major importance

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