Review

Exploring the genetic susceptibility of chronic widespread pain: the tender points in genetic association studies


Chronic widespread pain (CWP) is a prevalent disorder associated with a low pain threshold and increased levels of psychological distress. Evidence indicates that there is a genetic component to CWP syndromes and pain sensitivity. Here we have identified and reviewed the current literature on genetic association (GA) studies of CWP and pain sensitivity by searching MEDLINE and EMBASE between January 1990 and May 2007. Of the 18 candidate genes studied to date, no definitive susceptibility genes have been identified. This review highlights the key issues for consideration when interpreting the findings from existing studies and in designing future studies to ensure robust and comparable findings in this field. Well-designed GA studies are essential if the genetic component to CWP aetiology is to be fully determined.

**KEY WORDS:** Fibromyalgia, Chronic widespread pain, Pain sensitivity, Genetics, Study design.

**Introduction**

The term chronic non-inflammatory musculoskeletal pain captures a number of disorders that are common in their lack of a clear pathological aetiology. These are considered as either regional [including low back pain (LBP), knee, shoulder and neck pain] or widespread disorders. Of interest here is chronic widespread pain (CWP), defined by ACR as pain that involves two contralateral quadrants of the body and the axial skeleton that persists for 3 months or longer [1]. It has a prevalence of \~11% in the general population [1] and is the distinguishing feature of the fibromyalgia (FM) syndrome.

Despite the unclear aetiology of CWP and FM, an emerging evidence base has identified psychological and stress-related factors as important predictors of onset [2]. Whether these factors are moderated by a genetic susceptibility is unknown. A number of studies have repeatedly demonstrated the familial aggregation of FM [3–6]. Evidence from twin studies, although limited, supports a heritability component to CWP. Kato et al. [7] conducted a study of 4170 monozygotic (MZ), 5881 same-sex and 5755 opposite-sex dizygotic (DZ) twin pairs from the Swedish Twin Registry. The overall heritability estimates for CWP were reported to be 48–54% with no difference in type or size of genetic contribution noted between genders. This heritability component in CWP reflects that of regional pain disorders including LBP and neck pain (heritability estimates range from 52% to 68% and 35% to 68%, respectively) [8].

Classification criteria for FM require the presence of widespread tenderness, as measured by a high tender point count (≥11 of 18), in addition to the presence of CWP [1]. A tender point count, pain threshold and tolerance testing are commonly used methods to assess pain sensitivity. Subjects with CWP have lower pain thresholds [9] and higher tender point counts [9, 10] than those free of pain or with regional pain. Ethnic and gender differences in pain sensitivity [11] suggest that it may also be under genetic control. Given the available evidence and the close relationship with CWP, pain sensitivity renders itself as an important area of research when investigating a genetic susceptibility to CWP.

Genetic association (GA) studies allow the proposed genetic susceptibility of CWP and pain sensitivity to be explored by investigating the role of variation within candidate genes. Our aim was to assess the current knowledge in this field by performing a comprehensive narrative review of the current literature on GA studies for both CWP disorders and pain sensitivity and to discuss the findings in the context of study design issues in order to guide further research in this field.

**Methods**

MEDLINE and EMBASE (on OVID) were searched for publications using a combination of a pain outcome term (PAIN or CHRONIC WIDESPREAD PAIN or FIBROMYALGIA) and a genetics-related term (GENETIC or GENE or POLYMORPHISM or GENOTYPE or HAPLOTYPE or SNP or ALLELE or VARIANT) in the title in order to identify all GA publications. The search was limited to English language journals and human studies from January 1990 to May 2007. The reference lists in the relevant identified articles and previous pain and genetics review papers were also checked to identify further relevant papers. Study design information and results were extracted from each paper and the information was verified by a second independent reader.

**Discussion of findings**

A total of 207 papers were identified by the search, 20 of which were suitable for review. Unsuitable papers included studies on gene therapy \( (n = 30) \), review articles \( (n = 36) \), rare mutations/familial pain insensitivity syndromes \( (n = 18) \), twin/family studies \( (n = 9) \), other types of pain \( (n = 52) \) and inapplicable \( (n = 42) \). A further two relevant papers identified from a hand search were also reviewed. The 22 papers report on GA analysis with 18 genes in over 10 different study populations. The study population, markers, outcome of interest and findings from the GA studies for each gene are detailed separately for CWP/FM (Table 1) and pain sensitivity (Table 2).

The neurophysiology of pain involves a complex network of systems in both the peripheral and central nervous systems [12, 13], and in fact, the majority of neurotransmission pathways have been to some extent linked to pain. This has made the
potential candidate gene list extensive; however, the majority of genes studied are inter-linked. To date, 10 genes have been tested as candidates for CWP disorders and they have largely focused on aspects of neurotransmission, in particular, genes involved in the action of serotonin (5-HT), a neurotransmitter involved in the regulation of many bodily functions. Lower serum concentrations of 5-HT have been observed in patients with FM [14]. Genes investigated include the monoamine oxidase A (MAOA) gene, serotonin receptor genes (HTRA2, HTRA3 and HTRA3B) and a serotonin transporter gene (SLC6A4). MAOA degrades serotonin and its enzymatic activity has been shown to have high inter-individual variability. Consequently, the role of a synonymous SNP (single nucleotide polymorphism), rs6323 in the MAOA gene, which reportedly affects the activity of the enzyme [15], was investigated in FM; however, no association was seen in a Taiwanese population [16]. In HTRA2, a single synonymous SNP, T102C, has been studied. Bondy et al. [17] found the TT genotype of T102C to be less frequent in FM but conversely it resulted in higher pain scores. Gursoy et al. [18] reported no association with FM but a lower pain threshold in TT homozygotes. The HTRA3A and HTRA3B genes have been more comprehensively studied than HTRA2 but no association with FM has been observed [19]. In two case–control studies of FM the short allele (SS) genotype of the promoter region 44 bp indel (insertion/deletion) (also known as 5HTTPLR) in SLC6A4 was observed at a significantly higher frequency in FM [20, 21]. Contrary to this finding, Gursoy [22] reported no association between FM and the indel or a variable number tandem repeat (VNTR) polymorphism in SLC6A4.

Dopamine is an important neurohormone that has wide-ranging influences throughout the body. In an Israeli population, the dopamine receptor gene DRD4 was found to have a reduced frequency of the seven repeat allele of the 48 bp exon VNTR in patients with FM. This allele was also associated with increased novelty-seeking behaviour that is low in FM [23].

A number of inflammatory mediators: endothelial nitric oxide synthase (NOS3), SERPINAI (a protease inhibitor that protects tissues from the enzymes of inflammatory cells) and the anti-inflammatory cytokine IL4, have also been investigated as candidate genes for FM. An increased frequency of the SERPINAI PI’Z allele was observed in FM in a Spanish case-control study [24]. No association was observed between the single SNPs studied in IL4 and NOS3 with FM [16, 25].

To date, the most widely studied gene is COMT, which codes for catechol-O-methyltransferase, an enzyme that degrades catecholamine neurotransmitters, including dopamine, epinephrine and norepinephrine. The variant allele V158M results in reduced enzymatic activity due to its effect on thermobility [26] and has been associated with reduced μ-opioid activity in response to pain stimuli resulting in increased pain sensitivity [27]. The low (MM) and intermediate (VM) activity genotypes were significantly increased in frequency in FM patients in two case-control studies [28, 29], however, a much larger Norwegian cohort study (the HUNT study) found no association between the polymorphism and CWP [30].

Studies have also looked at the relationship between this SNP and others in the COMT gene with pain sensitivity. Diatchenko et al. [31] genotyped common SNPs across the gene in healthy women and identified a haplotype block that could discriminate individuals with low, average or high pain sensitivity and functional studies suggest that this is due to altering mRNA secondary structure [32]. They subsequently showed that the V158M associates with temporal summation of pain [33] and proposed differing roles for the variants in the gene on the membrane-bound and soluble forms of COMT. Kim et al. [34] also reported associations between SNPs in COMT and heat and cold pain sensitivities.

A further eight genes have also been tested for GA with pain sensitivity. GTP cyclohydrolase (GCH1), an enzyme involved in the production of tetrahydrobiopterin, a co-factor of enzymes involved in the synthesis of neurotransmitters including NO, is the most comprehensively studied of these genes. Testing for association between SNPs across GCH1 and post-operative pain identified multiple SNPs and a haplotype associated with lower
pain scores. Healthy individuals with this pain-protective haplotype showed reduced pain responses to a mechanical stimulus [35]. Kim and Dionne [36], however, subsequently found no association between haplotypes in GCH1 with cold or heat pain sensitivity in healthy individuals.

GA studies of pain sensitivity have considered the role of genetic variation in opioid receptors (OPRD1 and OPRM1) that have crucial roles in pain mediation. In OPRD1, a non-synonymous SNP was found to be a determinant of heat pain sensitivity [37]. In OPRM1, research has centred on the A118G polymorphism as the variant allele increases the protein’s binding sensitivity [37]. In Fillingim et al. [39] carrying the G allele was associated with reduced pain sensitivity. OPRM1 is involved in modulation of the hypothalamo-pituitary–adrenal (HPA) axis, the innate stress response axis, and the G allele of A118G SNP has also been associated with an increased cortisol response to opioid receptor blockage [40]. Individuals with non-synonymous variants in melanocortin-1 receptor (MC1R), a key hormone in stress response, which renders it non-functional, non-synonymous variants in melanocortin-1 receptor (MC1R), a key hormone in stress response, which renders it non-functional.

Kim et al. [34, 37] investigated GA with pain sensitivity in FAAH (breaks down a cannabinoid receptor agonist believed to have a role in pain perception) and TRPA1, TRPM8 and TRPV1 (ion channels involved in pain transmission, which are activated by different stimuli). SNPs and/or haplotypes in FAAH, TRPA1 and TRPV1 showed association with pain sensitivity in a healthy US Caucasian population.

Design considerations for GA studies of pain
Two key reviews by Cardon and Bell [42] and Cordell and Clayton [43] consider appropriate study design and the related issues when conducting a GA analysis. Some of these issues have also been discussed in the context of neuropathic pain (pain due to injury to the nervous system) research in Belfer et al. [44]. GA analysis is in its relative infancy in this field compared with other complex diseases, e.g. type II diabetes where expectations to fulfil these criteria are consequently higher. In order for us to have confidence in our findings, it is important that the guidelines adhered to in GA studies of other complex diseases are also followed when researching the genetics of chronic pain.

Ascertainment of pain phenotype
The ascertainment of the phenotype of interest can often be a major source of contention. GA studies of chronic pain involving FM have used the 1990 standard ACR criteria to classify cases [1]. The use of this standard classification allows for direct comparison of homogeneous groups with respect to their pain status. However, CWP relies on self-reported measures and in the past the ACR classification criteria has been criticized for being too inclusive [45] and stricter criteria may be more appropriate.
Pain sensitivity and tolerance are subjective measures and are difficult to ascertain. Numerous techniques, as recorded in Table 2, have been used to measure pain sensitivity, which has been demonstrated to have high inter-individual variation [31], but as of yet no ‘gold standard’ has been determined. Heat and cold stimuli are frequently used. Kim et al. [34, 37] asked healthy subjects to report the intensity of pain experienced when exposed to cold and thermal stimuli using a visual analogue scale (VAS). In contrast, Diatchenko et al. [31] derived their own unit measure of pain sensitivity using a summated Z-score for cutaneous and deep muscle pain based on pressure, thermal and ischaemic pain thresholds and tolerances. Similar methods were used in a study of GCH1 haplotypes in a group of healthy subjects, findings from which supported the group’s earlier work on persistent LBP following a discectomy [35].

**Choice of control group**

In case–control studies, the selection of control subjects is equally as important as the selection of cases. Controls must be eligible to become a case if they develop the disease of interest and therefore should be selected from the same source population as cases [46]. If cases, however, are taken from a specialist sample, such as a clinic population, then the best source of controls is less clear, although their eligibility to become a case should always remain. In general, in the GA studies of FM reviewed for this article, little information on controls has been provided.

**Candidate gene selection**

Candidate gene selection in the association studies thus far has been based on a strong biological rationale such as a role of the resulting protein in nociception. Experimental evidence is useful in prioritizing candidates and has also been a factor in the selection of many of the candidate genes chosen. The following criteria add weight to the hypothesis of a genetic effect. The first is location within a region of linkage for the desired outcome. To date, human linkage studies with pain outcomes have only investigated the HLA, SLC6A4 and HTR2A loci [47, 48], and quantitative trait loci (QTL) mapping using mice has identified MC1R [49] and OPRD1 [50]. The second is a previously reported GA between the gene and the desired or a related outcome, although this can be somewhat uninformative if previous studies were under-powered and negative results may not have been reported due to publication bias. The third is known functional polymorphism/s, e.g. A118G in OPRD1 and V158M in COMT have functional effects. The last is differential expression of the gene in pain phenotypes, which to date has been widely examined in animal models but not in humans [51] and gene knockouts or disruption in transgenic mice causing a pain phenotype [52]. Gene expression profiling and gene knockouts, however, tell us only that the gene functions in pain and does not inform about the effects of common variants within the gene on a pain outcome.

**Genetic marker selection**

A large proportion of the published GA analyses within this field have only tested for association with a SNP of known or purported function, e.g. the V158M SNP in COMT. This approach does not account for other variants within the gene or its regulatory regions, which may act synergistically with the known functional variant. It is, therefore, more useful to use a systematic approach whereby the common variation within the gene is examined by selecting markers for genotyping based on patterns of linkage disequilibrium (LD). With the role of the majority of SNPs being unknown this method allows identification of variants that may be important, e.g. due to location in unknown regulatory regions. Few of the studies reviewed here have used this approach but alternatively have genotyped common SNPs spaced across the gene of interest, which can subsequently be used to examine LD in the specific population. They have however, demonstrated the importance of looking at multiple SNPs in the gene. Diatchenko et al. [31] genotyped common SNPs across COMT and identified a haplotype block of four SNPs, including the functional SNP V158M, that consists of three common haplotypes. These were shown to be associated with pain sensitivity and COMT activity in vitro; however, this was not simply due to the functional SNP as it occurred in both high and low pain sensitivity haplotypes.

**Sample size and power**

Power is the most prevalent problem in the literature reviewed. If pain disorders are indeed polygenic, and affected by environmental influences, as they are proposed to be, then the risk of developing the disease conferred from a single variant or gene is likely to be very small and consequently a large sample size is required to detect it. To date most sample sizes used, as summarized in Table 1, have been insufficient to detect the reported effects, with many studies having <100 cases and 100 controls. For example, two studies testing for a GA between the V158M SNP in COMT and FM: Gursoy et al. [28] used 61 cases and 61 controls and García-Fructoso et al. [29] used 46 cases and 40 controls. A simple power calculation shows that to detect the difference in allele frequency reported between cases and controls in the two studies (~12%) ~250 cases and 250 controls would be required to have 80% power and 5% type I error. Other factors, however, such as lower allele frequency and testing multiple SNPs will increase sample size requirement further. In the case of chronic pain disorders, inadequate sample size may in part be due to their low prevalence (e.g. 2% for FM), which could make it more difficult to establish an appropriate study population. Power is greater when using a continuous outcome such as pain sensitivity rather than a binary outcome of a pain disorder. Many of the studies testing for associations between genes and pain sensitivity, however, are also likely to have been under-powered and larger groups of healthy volunteers are required to validate such findings, e.g. the relationship implicated between GCH1 and pain sensitivity [35], which was not replicated in a second study [36].

**Interpreting results from GA studies**

GA analysis is based on the assumption that if a variant within a gene contributes to a disease phenotype then it will be more prevalent in individuals with the phenotype. Current statistical methods for GA analysis of single SNPs and haplotypes are detailed in Balding [53]. Results from the reviewed GA studies need to be interpreted with caution as a significant association has three possible explanations: (i) it is a real association with the variant having a direct effect on the pain outcome, (ii) it is due to the associated polymorphism being in LD with the causual polymorphism or (iii) the association has occurred by chance. Chance findings may occur due to insufficient power (as previously discussed), population stratification, confounding or multiple testing.

**Population stratification**

Population stratification occurs when cases and controls have different allele frequencies due to diversity in the background population rather than association with disease [54]. Methodology studies have shown that population stratification will only bias the odds ratio in a case–control study where there is a large difference in genotype frequency between the admixed populations and that the overall bias from population stratification is small [55]. For example, Diatchenko et al. [31] tested for association between variants in COMT and pain sensitivity in an American population that is 85% Caucasian. They report no significant differences in the results when stratifying to Caucasians only. There are known
Confounding

Confounding has been somewhat overlooked in the GA studies of chronic pain disorders to date. FM, in particular, has repeatedly been demonstrated to be associated with high levels of psychological distress and depression, e.g. Benjamin et al. [59]. Many of the candidate genes studied thus far with FM have previously been associated with neuropsychiatric disorders such as anxiety and depression e.g. COMT, DRD4, HTR2A, MAOA and SLC6A4. Despite this, attempts to adjust, and therefore account for the influence of psychological status on the findings reported have been rare. In Cohen et al. [21] and Offenbaecher et al. [20] the SS (short allele) genotype for the 44 bp indel promoter polymorphism in the serotonin transporter gene, SLC6A4, was observed in increased prevalence in FM cases. Offenbaecher et al. [20] observed a trend for increased frequency of the S allele after removing individuals with high scores on Beck’s Depression Index (BDI) and the Symptoms Checklist SCL-90R, a multi-domain measure of psychological distress, from the analysis. There was a strong association of depression and distress with FM, therefore the number of cases was reduced from 62 to 20 and 18 for each analysis, respectively resulting in low statistical power [20]. In contrast, Cohen et al. [21] found that adjusting for psychological factors rendered the association non-significant. Gursoy et al. [22] also found that there was no association between the indel and FM in mentally healthy individuals. The S allele of this indel has previously been associated with anxiety-related personality traits [60] and the literature suggests that the association observed in FM may be due to the confounding effect of psychological morbidities.

A number of other factors are known to be associated with both chronic pain and genetics; these include gender, ethnicity, drug use and menstruation, all of which may act as potential confounders to any GA observed.

Multiple testing

Multiple testing must also be considered when interpreting results; this is also uncommon in the reviewed literature, yet is a requirement for a high-quality GA study, particularly in studies examining novel candidates or a large number of variants. Bonferroni correction is often used but can be too conservative when linked variants are being tested. More appropriate methods include generating an empirical P-value using permutations or calculating false discovery rate [53].

Replication of findings

If an association is interpreted as real, then the true test is whether or not it replicates in an independent data set. Only 7 of the 18 genes considered here have been analysed in multiple publications. They are often heterogeneous in nature with different outcomes, differing ethnicity and different markers being genotyped and therefore there are only a few examples where the data are comparable. For the COMT gene two studies have observed an increased frequency of the M allele of the V158M polymorphism in FM cases in Turkish [28] and Spanish populations [29]. Although this may be deemed a replication, the sample size used in both studies was small and a larger study is required to confirm the association.

Kim and Dionne [36] recently attempted to replicate the findings of Tegeder et al. [35] of an association between a pain-protective haplotype of GCH1 and pain sensitivity, without success. There are, however, a number of reasons why this may be the case. First, the studies report differing haplotype block structures despite both being American Caucasian populations. Second, the outcomes measured are also different with Tegeder et al. [35] using Z-scores for thermal, mechanical and ischaemic pain tolerance and Kim and Dionne [36] using VAS scores for pain sensitivity to hot and cold stimuli and finally despite seeing a trend with thermal pain, Tegeder et al. [35] did not report a significant association within their 547 healthy volunteers, only with mechanical pain. It is not unsurprising then that Kim and Dionne [36] failed to replicate the result in their sample of 368 Caucasians. They also stratified their analysis by gender, further reducing sample size and power. Replication studies have a tendency to show smaller effect sizes than initial studies reporting a GA [61], therefore, replication studies require a larger sample size to detect the same association.

Concluding remarks

No definitive pain susceptibility genes have yet been identified but the field is in its relative infancy compared with many complex diseases. However, many of the genes reviewed here warrant further investigation. The existing studies are subject to many study design issues. Careful consideration needs to be given to these issues when interpreting findings from existing studies and when designing future studies into the genetic susceptibility of CWP and pain sensitivity, in order for reported findings to be as robust as possible.

We conclude that future GA studies of chronic pain disorders should have adequate sample size for sufficient power to detect associations. Candidate gene selection should be based on strong biological rationale with supporting evidence from experimental and genetic studies and methods that capture the variation within the genes of interest based on LD should be used. Appropriate information on pain status and potential environmental and psychological confounders is also required. Finally, significant associations must be replicated in an independent data set. This type of study is essential if the genetic component to CWP aetiology is to be fully elucidated.

Rheumatology key message

- The susceptibility genes for CWP syndromes have not been identified partly due to poor study design, which should be noted when interpreting existing findings and addressed when designing future studies.

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References

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