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Genomic loci and candidate genes underlying inflammatory nociception

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Abstract

Heritable genetic factors contribute significantly to inflammatory nociception. To determine candidate genes underlying inflammatory nociception, the current study used a mouse model of abdominal inflammatory pain. BXD recombinant inbred (RI) mouse strains were administered the intraperitoneal (IP) acetic acid test and genome-wide quantitative trait locus (QTL) mapping was performed on the mean number of abdominal contraction and extension movements in three distinct groups of BXD RI mouse strains in two separate experiments. Combined mapping results detected two QTLs on chromosomes (Chr) 3 and 10 across experiments and groups of mice; an additional sex-specific QTL was detected on Chr 16. The results replicate previous findings of a significant QTL, *Nociq2*, on distal Chr 10 for formalin-induced inflammatory nociception and will aid in identification of the underlying candidate genes. Comparisons of sensitivity to IP acetic acid in BXD RI mouse strains with microarray mRNA transcript expression profiles in specific brain areas detected covarying expression of candidate genes that are also found in the detected QTL confidence intervals. The results indicate that common and distinct genetic mechanisms underlie heritable sensitivity to diverse inflammatory insults, and provide a discrete set of high priority candidate genes to investigate further in rodents and human association studies.

Keywords

inflammation; nociception; acetic acid; quantitative trait locus mapping; microarray; transcript abundance

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Conflict of Interest Statement

The authors have no conflicts of interest to report.

1. Introduction

Understanding what predisposes patients to increased inflammatory pain sensitivity may improve prediction and prevention of suffering and improve patient care. Rodent studies have demonstrated that genetic background significantly affects sensitivity to a number of inflammatory insults [23,52]. Although many genes have been implicated in the modulation of inflammatory nociception using knockout mouse and other techniques [20], the genes responsible for heritable individual differences are largely unknown. Quantitative trait locus (QTL) mapping of somatic inflammatory nociception in the intraplantar formalin test has detected two genomic loci, *Nociq1* and *Nociq2*, linked to heritable differences on chromosomes (Chr) 9 and 10 of the mouse, respectively [52]. Analysis of congenic mouse strains has detected a third QTL on Chr 12, *Nociq3* [32]. Within these genomic regions are candidate genes that underlie heritable variability in inflammatory nociception. The candidate gene, *Atp1b3*, has been identified as likely responsible for *Nociq1* and sensitivity in the early phase of the biphasic formalin response of paw licking [21]. The responsible candidate genes underlying the second, late phase of formalin responding traditionally ascribed more to tissue inflammation than the early phase [47] remain to be identified.

Visceral inflammatory pain or nociception and the underlying mechanisms differ in significant ways from somatic inflammatory pain or nociception [38]. The precise molecular mechanisms that underlie these differences are still under investigation. Previous findings, however, also suggest that sensitivity to somatic and certain visceral inflammatory insults share underlying genetic mechanisms as they are genetically correlated in standard inbred strains of mice [23,33]. Standard inbred mouse strains that are more sensitive to subcutaneous (SC) injection of formalin, bee venom or capsaicin are also more sensitive to intraperitoneal (IP) injection of acetic acid or magnesium sulfate; Spearman rank correlation coefficients between spontaneous inflammatory nociceptive behaviors in these somatic and visceral models range from 0.41 – 0.79, with a mean and median of 0.64 [23,33]. These genetic correlations indicate that common genetic mechanisms are shared among the pain models [18], despite differences in the inflammatory irritant, the site of administration, and the behavioral response [33]. Identification of the common mechanisms among these pain models will help prioritize the search for the most broadly acting candidate genes for inflammatory nociception and pain.

The current study examines the genetics of inflammatory nociception using QTL mapping to detect regions of the mouse genome and candidate genes linked with sensitivity to IP injection of acetic acid in multiple panels of recombinant inbred (RI) strains of mice. In addition, genetic correlation analysis of acetic acid-induced responding with tissue-specific transcript expression profiles is performed to obtain convergent evidence for candidate genes found in the detected QTLs.

2. Methods

2.1. Subjects

Mice of C57BL/6J, DBA/2J and 24 BXD RI (all “TyJ”) strains were used in the experiments. BXD RI mice have been created by crossing the standard inbred strains C57BL/6J and DBA/2J and re-inbreeding the F₂ hybrid offspring [45]; they have been maintained inbred since their creation. These RI strains represent a genetic reference population with a fixed genotype for which genotype, phenotype, and transcript data collected over time and across experiments are directly comparable [9].

Mice were tested in two separate experiments performed independently and at different times and locations. This provided the opportunity to quantitatively test for convergence of

findings from two distinct experiments with the same hypothesis. The HHF and HHM groups of mice were tested at the Portland Veterans Affairs Medical Center Veterinary Medical Unit by author H.H. The RMQ group of mice was tested at the University of Illinois College of Medicine at Rockford by author R.M.Q. as part of a previously published report [37]. All procedures were approved by the IACUC of the institutions and all experiments adhered to the guidelines of the Committee for Research and Ethical Issues of the IASP [53].

Male mice in the RMQ group were obtained from The Jackson Laboratory (JAX, Bar Harbor, Maine) and tested at least one week after arrival ($n = 4\text{--}10/\text{strain}$; $N = 122$). Mice from the HHF and HHM groups were female and male offspring, respectively, from matings of mice of the same strain obtained from JAX and tested after at least one week of acclimation to the final housing facility ($n = 6\text{--}13/\text{strain/sex}$; $N = 240$ for HHF, 233 for HHM). The sexes were separated at weaning at 21–25 days of age and housed 1–4 mice per shoebox cage. Single housing was avoided when possible and testing was avoided for 24 h after a clean cage change. Food and water were available *ad libitum*. The colony rooms were on a 12 h light/dark cycle. All mice were at least six weeks of age when tested. HHF and HHM mice were previously tested for analgesia in the acetic acid test seven to nine days prior. Testing seven to ten days prior does not have a significant effect on responses in the acetic acid test [37].

2.2 Acetic acid inflammatory nociception assay

For the HHF and HHM groups, glacial acetic acid (EM Science, Gibbstown, NJ) was diluted with 0.9% saline to a final concentration of 0.65% made fresh each week. For the RMQ group, a final concentration of 0.6% acetic acid solution was used. All mice were acclimated to the procedure room for at least one hour prior to testing. Testing was done between the hours of 10:00 and 16:00. HHF and HHM groups of mice were placed in Plexiglas® cylinders (29 cm×30 cm diameter) on a counter top for 30 min, injected with saline (SC, 10 ml/kg; vehicle injection for separate analgesia study), and 20 min later acetic acid was injected (IP, 10 ml/kg). HHF and HHM mice were placed in the cylinders and observed continuously for 30 min after injection of the acetic acid for the number of abdominal contraction and extension movements. These abdominal extensions (also referred to as ‘writhes’), defined as lengthwise constrictions of the torso with a concomitant concave arching of the back, are quite stereotypical and are easily distinguished from other behaviors. One to four mice were observed and scored at a time, which was possible because a single response lasts several seconds and the frequency is low, on average $< 2/\text{min}$. Up to five sets of four mice were observed per day. RMQ mice were observed and scored continuously for six minutes beginning five minutes after acetic acid injection (i.e. 5–11 min after injection). The slight differences in parameters used for HHF, HHM and RMQ groups do not preclude combination of the data with Fisher’s method described below in section 2.5. [44]. In fact, the current study is partly intended to determine linkage of genomic regions and candidate genes to inflammatory nociception despite methodological differences (including, more broadly, differences between acetic acid and formalin assays).

2.3. Statistical analysis of strain differences and heritability estimates

The effect of BXD RI mouse strain on mean number of abdominal extensions was determined by a one-way analysis of variance (ANOVA). Heritability was determined as $h^2 = V_A/(V_A + V_E)$, where V_A is the additive genetic variation estimated by the between-strain variance and V_E is the environmental variance estimated by the within-strain variance from the ANOVA results [14]. Note that the additive genetic variance, when estimated using isogenic BXD RI strains, also contains gene-by-gene and gene-by-environment interactions, and that environmental variance is confounded with any technical variation. This estimate

does not directly estimate the transmission of phenotypic values from parent to offspring, but provides an estimate of the resemblance among isogenic relatives.

2.4. Quantitative trait locus (QTL) mapping

To determine regions of the genome containing polymorphisms of relevance to variability in inflammatory nociception, whole-genome single locus QTL mapping of the BXD strain means was performed for each of the groups, HHF, HHM and RMQ. DNA marker-trait associations were assessed by regression analysis using the QTL detection software package, Map Manager QTX [27,28] as integrated with the GeneNetwork/WebQTL website (www.genenetwork.org; www.webqtl.org) [51]. Interval maps of the associations across the genome were constructed using the online software WebQTL (www.genenetwork.org) [4,51]. A total of 3795 non-redundant DNA markers polymorphic between the parental C57BL/6J and DBA/2J strains were considered (June 2005 freeze; www.genenetwork.org/dbdoc/BXDGeno.html). The threshold logarithm of the odds (LOD) score for a significant QTL was determined using an empirical p -value from 1000 permutations of the strain means with a genome-wide error rate $\alpha = 0.05$ [7].

When two loci were detected in close proximity to each other, multiple-QTL modeling was performed to determine whether the loci are independent of one another in explaining variation in mean responses to IP acetic acid. Multiple QTL model fitting was performed using functions available in the R/QTL package (R version 2.11.1, R/QTL 1.18–7). An additive model incorporating the two loci was fit and p -values for individual model terms were obtained by dropping them one at a time. This determines the relative importance of the individual terms in the model and determines whether a more parsimonious single QTL model is sufficient to explain the observed phenotypic variance.

2.5. Fisher's method of combining results of multiple related experiments

Fisher's method of combining probabilities of multiple independent experiments permits the statistical results of highly related experiments testing the same hypothesis to be combined quantitatively [44]. This method has been used successfully to determine the robustness of results in replicate QTL mapping studies for other pain traits including swim stress-induced analgesia [31]. Note that slight differences in experimental design are acceptable with this approach [44], and hence, the slight differences between experiments in acetic acid concentration used (0.6% or 0.65%) and total time of the observation period (6 or 30 min) do not preclude use of the method. In fact, for the current study, the method permits the determination of QTL mapping results that are robust to these slight differences, and thus, highly unlikely to be due to chance. For each of the HHF, HHM, and RMQ groups, the p -value of the Pearson product-moment correlation coefficient between DNA marker values (0 or 1 for C57BL/6J or DBA/2J homozygotes, respectively) and strain means of number of abdominal extensions was calculated for each marker. For each marker, the p -value results from the three mapping populations, the HHF, HHM, and RMQ groups, were combined using the formula $\chi^2 = -2\sum \ln p$. This χ^2 value is then compared to an inverse χ^2 distribution with $2(3 \text{ independent tests}) = 6$ degrees of freedom (df) to yield a combined p -value. Bonferroni correction for the 3795 genome-wide comparisons renders the critical p -value $(0.05/3795) = 1.32 \times 10^{-5}$, which corresponds to a critical χ^2 value of 32.48 with 6 df .

2.6. Transcript covariance analysis

Transcript covariance with the trait provides an indication of the momentary effective genotype or transcriptional context in which sensitivity to a trait is determined [29] and can be used to empirically reduce the list of candidate genes from QTL mapping results [12]. Genetic correlation analysis was performed against microarray measures of transcript abundance to determine which transcripts' expression covaries with strain means of

sensitivity to acetic acid. Because the BXD RI strains of mice are a genetic reference population with fixed, segregated genotypes, phenotypic measurements can be combined over time and across studies. High positive correlations between acetic acid responding and gene expression for the same strains of mice indicate shared genetic mechanisms between the trait and the gene's expression [9,18]. Basal tissue-specific mRNA transcript expression levels have been determined with microarray methods for several brain areas of up to 67 BXD RI strains, including the 24 strains tested here, and are publicly available on the GeneNetwork website (www.genenetwork.org) [4,51]. Pearson product-moment correlations were calculated between strain means of abdominal extensions and transcript expression values in publicly available Affymetrix or Illumina genome-wide mRNA microarray data for the cerebellum, hippocampus, whole neocortex, prefrontal cortex, nucleus accumbens, and the striatum of BXD RI strains [3,5,6,35]. These brain areas have been implicated in the modulation of sensory processes and responses to analgesic treatments, including from human brain imaging studies [1,2,13,17,40,46]. The data sets and complete methodological details for each microarray study are available on the GeneNetwork website (www.genenetwork.org). All samples were obtained from experimentally naïve adult mice. Comparisons were made with the most recent version of the microarray data for each tissue with RMA normalization when multiple normalizations were available. With the goal of prioritization of candidate genes identified with QTL mapping, a conservative critical $p = 0.0001$ was chosen as this provides a reasonable, expected range of number of correlated transcripts for a single brain area based on previous whole genome transcript expression studies of sensory traits [8,16] and has been shown to identify results that are reproducible with other molecular methods [36].

3. Results

3.1. Strain differences and heritability estimates

A wide range of sensitivity to IP acetic acid was observed among BXD RI mouse strains with 4.65, 6.12 and 5.05 fold ranges observed in mean responses in the HHF, HHM and RMQ groups, respectively (Fig. 1). Overall, there was a significant effect of group on responses ($F_{2,531} = 84.42, p < 0.0001$) and a significant effect of strain in the RMQ group ($F_{21,100} = 6.25, p < 0.0001$). Considering only the HHF and HHM groups, there was only a tendency toward a significant interaction of sex $\times$ strain ($F_{24,411} = 1.38, p = 0.11$), but a significant effect of sex ($F_{1,410} = 37.98, p < 0.0001$) and a significant effect of BXD RI strain ($F_{24,410} = 4.27, p < 0.0001$). Heritability estimates indicate that strain differences account for 31%, 25% and 57% of the variation in the HHF, HHM and RMQ groups, respectively.

3.2. QTL mapping

The interval maps in Figs. 2–4 show genomic regions linked to inflammatory nociception in the IP acetic acid assay. For the individual groups, a statistically significant QTL was detected on proximal Chr 16 (HHM group) and four suggestive QTLs were detected: one on Chr 16 just proximal to the significant QTL on Chr 16 (HHM group), one on mid-Chr 3 (RMQ group), and two on distal Chr 10 (HHM and RMQ groups) (Figs. 2–4). Fisher's method was applied to combine the results of the three groups and test whether individual group results that reached the level of statistically suggestive were consistently observed across groups and thus unlikely due to chance. Fisher's method detected statistically significant linkage on mid-Chr 3 and distal Chr 10 and a third QTL slightly below significance just proximal to the significant QTL on Chr 10 (Figs. 2 and 3). These QTL regions contain polymorphic genes responsible for variation in sensitivity to inflammatory nociception evoked by IP acetic acid. The regions of the significant QTLs on Chr 3 (54.93–60.47 Mb; Fig. 2), Chr 10 (119.90–122.07 Mb; Fig. 3) and Chr 16 (19.92–22.81 Mb; Fig. 4)

contain 26, 12 and 33 known candidate genes, respectively (Table 1) using a 1.5 LOD drop-off from the peak of the QTL or a $10^{1.5}$ -fold drop-off of Fisher's combined p -value (with χ^2 distribution). Note that a 1.5 LOD drop-off was used to determine confidence intervals as the conventional 1.0 LOD drop-off [22] has been criticized as biased, overly conservative, and possibly excluding true candidate genes [25,26,49,50].

Multiple QTL model fitting was performed to determine whether the proximal locus (at DNA marker *rs13480775*, 82.5 cM, 115.206 Mb) and distal locus (at *rs13480796*, 93.0 cM, 121.228 Mb) on Chr 10 are independent of one another in explaining variation in acetic acid-induced abdominal extension behavior in the BXD HHM group. The additive model containing the two loci on Chr 10 had a significant LOD score of 3.75 ($p < 0.01$) and the variance accounted for (VAF) was 51.3% of the total trait variance. Dropping *rs13480775* from the model accounted for significantly less variance than the full model (LOD score drop of 1.57; $\chi^2_{df=1} = 7.23$, $p < 0.05$) as *rs13480775* accounts for 34.3% of the phenotypic variance. Dropping *rs13480796* from the model did not significantly affect the LOD score (drop of 0.04; $\chi^2_{df=1} = 0.18$, $p = 0.67$). Thus, while the proximal locus is sufficient to account for the phenotypic variation in the BXD HHM data, the inclusion of the distal locus does not significantly improve the fit of the model. Note that even though a single locus model including only the distal locus explains a significant amount of variability in the HHM group (LOD = 2.19; $p < 0.01$; VAF = 34.3%), and Fisher's method detected a statistically significant locus at the distal marker, overall, it cannot be concluded that the proximal and distal loci on Chr 10 are independent of each other.

A similar approach was used to determine whether the two loci on Chr 16, at markers *rs4165069* (9.90 cM, 17.57 Mb) and *rs4165279* (12.01 cM, 21.24 Mb), are independent of each other in explaining variation in the BXD HHM group. The additive model containing both loci had a significant LOD score of 4.64 ($p < 0.001$) and accounted for 58.9% of the trait variance. Dropping *rs4165069* from the model did not significantly affect the LOD score (drop of 0.07; $\chi^2_{df=1} = 0.34$, $p = 0.84$), and dropping *rs4165279* resulted in a LOD score decrease of 1.16 ($\chi^2_{df=1} = 5.33$, $p = 0.07$). Thus, whereas inclusion of the proximal locus does not significantly improve the fit of the model, it cannot be concluded that the proximal and distal loci on Chr 16 are independent of each other.

3.3. Transcript covariance analysis and convergence with QTL mapping results

Thirty-one transcripts had high abundance covariance with acetic acid response means ($|r| \geq 0.6$, $p < 0.0001$) in two or more of the HHF, HHM or RQM BXD RI groups (Table 2). Of particular interest are the genes *Dclk1*, *Eif2a*, and *Wwtr1*, which are the only genes with high transcript covariance and that are also found in the detected QTLs, in this case all on Chr 3 (Tables 2 and 3). Expression of *Dclk1* in the prefrontal cortex is highly negatively correlated with response means of all three groups ($-0.73 < r < -0.64$), and expression in the nucleus accumbens is highly negatively correlated with response means of two of the groups ($-0.80 < r < -0.66$) (Table 3). Expression of *Eif2a* in the prefrontal cortex is highly negatively correlated with response means of all three groups ($-0.79 < r < -0.68$) and expression of *Wwtr1* in the striatum is highly negatively correlated with response means of two of the groups ($-0.75 < r < -0.61$) (Table 3).

4. Discussion

As for many other sensory, nociceptive and antinociceptive traits examined previously in inbred mouse strain panels, sensitivity to IP acetic acid shows marked strain differences and sufficient heritability in the BXD RI mice to merit further investigation into, and identification of, the underlying genetic mechanisms. Whole-genome QTL mapping detected genomic loci containing high priority candidate genes for inflammatory

nociception, including a novel locus on Chr 3, loci on Chr 10 that overlap with a previously detected region, and a novel sex-specific locus on Chr 16.

Significant linkage was detected between acetic acid induced inflammatory nociception and a locus on Chr 3 that showed highly similar and consistent mapping results across all three groups of BXD mice (HHF, HHM and RMQ). This QTL peak is distinct from those previously identified for other sensory traits and includes several genes for P2Y receptors (*P2ry12*, *P2ry13* and *P2ry14*) recently implicated in inflammatory pain models [24], and other G protein coupled receptors. However, a comparison with gene expression data in the BXD RI strains provides convergent evidence for other candidate genes in the region. Three candidate genes located in the QTL on Chr 3 also show high covariation of transcript expression profiles in the prefrontal cortex, nucleus accumbens or striatum with acetic acid responding in two or more groups of BXD RI mice: *Dclk1*, which encodes doublecortin-like kinase 1; *Eif2a*, encoding for eukaryotic translation initiation factor 2a; and *Wwtr1*, encoding for WW domain containing transcription regulator 1. A recent report has shown that inflammatory nociception induced by subcutaneous injection of paraformaldehyde in the rat hind paw increases the number of neurons with immunoreactivity to phosphorylated doublecortin-like kinase (pDCLK) and the specific signal density for pDCLK in the non-preganglionic Edinger-Westphal nucleus [39]. Although the 14-3-3 family of proteins, of which *Wwtr1* encodes a binding protein, have recently been implicated in inflammatory processes [54], and *Eif2a* has a recognized role in cellular stress responses [34], at present, it is not known what role these genes play in visceral inflammatory nociception. Thus, although the convergent QTL mapping and expression findings suggest that these genes merit further study for possible roles in inflammatory nociception, these or other candidate genes in the linked region may be responsible for the QTL on Chr 3.

Based on quantitative combination of the results of the three BXD groups (with Fisher's method), a locus on distal Chr 10 was detected with significant linkage to acetic acid responses and a second slightly more proximal locus was found very close to significantly linked and may be male-specific. These QTLs are in the same region as *Nociq2*, the QTL previously linked to murine sensitivity in the early and late phases of the intraplantar formalin test of inflammatory nociception [52]. Although the current results may appear to resolve *Nociq2* into two loci as previously suspected but not concluded upon [52], multiple QTL modeling performed in the current study does not permit the conclusion that there are two independent loci. The loci cannot be quantitatively distinguished, due to linkage between the loci in the BXD RI strains, but it also cannot be concluded that the two loci are in fact a single locus. Thus, further analysis is necessary to determine if, in fact, there are at least two candidate genes underlying inflammatory nociception in the distal portion of Chr 10. The candidate genes in the more distal significant QTL are listed in Table 1. *Wif1* is a candidate gene located in the more distal locus that encodes for Wnt inhibitory factor 1 that might play a significant role in inflammatory nociception as Wnt signaling pathways have been implicated in inflammatory bowel disease and rheumatoid arthritis, and they show activation by inflammation and, in turn, upregulate pro-inflammatory genes [15,43,48]. *Avpr1a* might also play a role in inflammatory nociception as the arginine vasopressin (AVP) receptor encoded for by *Avpr1a* has been shown to mediate analgesia activated by endogenous ligands of the receptor [19,41]. Candidate genes responsible for the significant QTL are currently under investigation by us and others.

Consistent with previous reports of outbred mouse strains [30] and for inflammatory nociception [52], in the current study females were slightly to several fold more sensitive than males in the vast majority of BXD strains. In only two BXD strains were females at least slightly, but not significantly, less sensitive than males. The current study detected novel, sex-specific linkage of inflammatory nociception to a region of Chr 16. This region

shows statistically significant linkage in the HHM group of male BXD mice but not in the HHF group of female mice of the same experiment. Note that the RMQ group of male mice shows a similar mapping pattern that may not have been adequately sensitive to reach statistically significant levels possibly due to the truncated observation period of this group. At present, convergent evidence for specific candidate genes in the significant QTL peak on Chr 16 is not yet available, and thus it remains to be determined which candidate gene is responsible for the QTL.

Convergent prior evidence does exist for the candidate gene *Comt1* located at 18.407–18.427 Mb in the more proximal, male-specific, statistically suggestive QTL peak on Chr 16 reported here (Fig. 4). *Comt1* encodes the enzyme catechol-O-methyltransferase (COMT) that metabolizes catecholamines including epinephrine, norepinephrine and dopamine. In humans, the genotype (haplotype) for *COMT* associated with decreased COMT activity has been associated with increased pain sensitivity to noxious experimental stimuli and increased risk for temporomandibular joint pain disorder [10,11]. In rodents, inhibitors of COMT enzyme activity increase nociceptive sensitivity in rodents [11]. We recently reported that the genotype and expression of *Comt1* in standard inbred strains of mice is predictive of sensitivity in six assays of inflammatory nociception, including IP injection of acetic acid or magnesium sulfate, and subcutaneous injection of formalin, capsaicin, or bee venom [42]. Specifically, standard inbred strains such as the BXD progenitor strain DBA/2J that lack a novel B2 short interspersed nuclear element (SINE) found in the 3' UTR region of *Comt1* are more sensitive to inflammatory nociception (but not inflammation-induced hypersensitivity) than strains with the SINE element including C57BL/6J [42], due to a premature polyadenylation signal and increased COMT protein expression [42]. The genotype of BXD RI strains at this precise locus is not yet known. Overall, *Comt1* or other candidate genes in the suggestive and significant QTLs on Chr 16 may represent mechanisms that modulate inflammatory nociception only in males and thus may indirectly underlie the increased sensitivity of females to inflammatory nociception.

The current study represents only the second genome-wide study of genetic mechanisms underlying heritable variability in sensitivity to inflammatory nociception. The results confirm earlier findings indicating shared genetic mechanisms between the assays of formalin-induced and acetic acid-induced inflammatory nociceptive behavior in standard inbred mice [23,33] and provide specific potential candidate genes for subsequent investigation in rodents and human association studies. *Nociq2*, the QTL for formalin-induced inflammatory nociception, has been replicated and may still be two loci with more than one candidate gene on distal Chr 10 underlying inflammatory nociception. In addition, novel regions of Chr 3 and Chr 16 have been found to be linked to inflammatory nociception with convergent evidence for several candidate genes in the regions. Finally, sex-specific linkage was detected on Chr 16 and may reveal mechanisms that differ between the sexes and provide sex-specific therapeutic targets. Additional studies are necessary to determine which of the identified candidate genes are responsible for the current results.

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Figure 1.

Mean number of responses of BXD RI strains to IP acetic acid injection 0–30 min (HHF and HHM) or 6–11 min after injection (RMQ). BXD RI strain had a significant effect on responses ($p < 0.0001$). There was a significant effect of group (HHF, HHM and RMQ; $p < 0.0001$) with the RMQ group displaying the least responses and female mice (HHF) responding more than males observed for the same period of time (HHM).

Figure 2.

Interval mapping of acetic acid response strain means on *Mus musculus* chromosome 3 (only 40–80 Mb from centromere shown). LOD scores for the genotype-phenotype associations for each group are indicated by the red (HHF), blue (HHM), and green (RMQ) traces using the right y-axis. Permutation-based suggestive LOD score thresholds are indicated by the dotted line of corresponding color determined for each group based on 1000 permutations of the strain means of the group. A statistically significant QTL was detected at 54.93–60.47 Mb for the combined results of the individual groups using Fisher's method and Bonferroni correction (left y-axis, critical χ^2 value of 32.48 with 6 *df*, $p = 1.32 \times 10^{-5}$, indicated by dotted black line). Points on line for Fisher's combined *p*-value indicate location of DNA marker.

Figure 3.

Interval mapping of acetic acid response means on chromosome 10 (only 94–128 Mb from centromere shown). A statistically significant QTL was detected at 119.90–122.07 Mb for the combined results of the individual groups using Fisher's method (black trace; left y-axis). A second QTL slightly below significance was detected just proximal to the significant QTL. See Fig. 2 legend for more information.

Figure 4.

Interval mapping of acetic acid response means on chromosome 16 (only 5–35 Mb from centromere shown). Suggestive LOD score thresholds are indicated by the dotted line of corresponding color determined for each group based on 1000 permutations of the strain means of the group; the upper dotted blue line indicates the statistically significant LOD score threshold for the HHM group. Fisher's method did not detect a significant QTL for the combined results of the individual groups. A significant QTL was detected for the HHM group at 19.92–22.81 Mb that was not detected in the HHF group. See Fig. 2 legend for more information.

Table 1

Known candidate genes in confidence intervals of significant QTLs for acetic acid-induced inflammatory nociception. Genes indicated with bold font also show high transcript covariance with acetic acid response means (see Tables 2 and 3).

Chr 3 (54.93–60.47 Mb)
<i>Spg20</i>
<i>Sohlh2</i>
<i>Dclk1</i>
<i>Nbea</i>
<i>Mab2l1l</i>
<i>Tm4sf1</i>
<i>Tm4sf4</i>
<i>Wwtr1</i>
<i>Commd2</i>
<i>Gm410</i>
<i>Rnf13</i>
<i>Pfn2</i>
<i>Tsc22d2</i>
<i>Gdap9</i>
<i>Eif2a</i>
<i>Siah2</i>
<i>Clrn1</i>
<i>Gpr171</i>
<i>P2ry14</i>
<i>Gpr87</i>
<i>P2ry13</i>
<i>P2ry12</i>
<i>Igsf10</i>
<i>Aadac</i>
<i>Sucnr1</i>
<i>Mbnl1</i>
Chr 10 (119.90–122.07 Mb)
<i>Hmga2</i>
<i>Msrb3</i>
<i>Lemd3</i>
<i>Wif1</i>
<i>Tbc1d30</i>
<i>Gns</i>
<i>Rassf3</i>
<i>Tbkl</i>

*Xpot**Srgap1**Tmem5**Avpr1a*

Chr 16
(HHM only)
(19.92–22.81 Mb)

*Klhl6**Klhl24**Yeats2**Map6d1**Par1**Cyp2ab1**Abcc5**Eif2b5**Dvl3**Ap2m1**Abcf3**Alg3**Ece2**Camk2n2**Psm2**Eif4g1**Clcn2**Polr2h**Thpo**Chrd**Ephb3**Magel1**Vps8**Ehhadh**Tmem41a**Liph**Map3k13**Senp2**Igf2bp2**Sfrs10**Etv5**Dgkg**Crygs**Tbccd1*

Table 2

Genes showing high transcript covariance with acetic acid response means ($|r| \geq 0.6$ with indicated groups, $p < 0.0001$ for one or more groups). Genes indicated with bold font are also found in the significant QTLs shown in Figures 2–4.

Brain Area (Strains in common)	HHF + HHM + RMQ	HHM + RMQ	HHF + RMQ	HHF + HHM
Cerebellum ^a				
Hippocampus ^b		<i>Eef1g</i>	<i>Adk5</i>	
		<i>Lbp</i>	<i>Gab1</i>	
			<i>Noc4</i>	
Neocortex ^c		<i>Cdcc122</i>	<i>Igf1bp4</i>	<i>Dph5</i>
			<i>Usp12</i>	<i>Man2b1</i>
				<i>Slc41a2</i>
Nucleus accumbens ^d		<i>Bace</i>	<i>Dcl1</i>	
		<i>Chmp4b</i>		
		<i>Dpcd</i>		
		<i>Ewsr1</i>		
		<i>Map2k6</i>		
		<i>Nat11</i>		
		<i>Pomp</i>		
		<i>Rbx1</i>		
		<i>Terf1</i>		
		<i>Timm17a</i>		
		<i>Zfp358</i>		
Prefrontal cortex ^e	<i>Dcl1</i>	<i>Mfap3</i>	<i>Asb1</i>	
	<i>Dusp12</i>		<i>Cyyr1</i>	
	<i>Eif2a</i>			
Striatum ^{f,*}			<i>Fancd2</i> ^{HB*}	
			<i>Ssh3</i> ^{HB*}	
			<i>Wwtr1</i> ^{HQF*}	

^aGE-NIAAA Cerebellum mRNA M430v2 (May05) RMA and SJUT Cerebellum mRNA M430 (Mar05) RMA microarray datasets;

^bHippocampus Consortium M430v2 (Jun06) RMA dataset;

^cHQF BXD Neocortex ILM6v1.1 (Feb08) RankInv dataset;

^dVCU BXD NA Sal M430 2.0 (Oct07) RMA dataset;

^eVCU BXD PFC Sal M430 2.0 (Dec06) RMA;

^{f,*}HQF Striatum Exon (Feb09) RMA and HBP Rosen Striatum M430V2 (Apr05) RMA Clean datasets. See www.genenetwork.org for complete details of each dataset.

Table 3

Correlations and *p*-values of candidate genes for inflammatory nociception found in significant QTL regions (indicated in bold in Tables 1 and 2) showing high transcript expression covariance with acetic acid response means.

Gene Symbol	Gene Name	Chr	Mb range	Brain Area	Pearson Correlation			Correlation <i>p</i> -value		
					HHF	HHM	RMQ	HHF	HHM	RMQ
Delk1	Doublecortin-like kinase 1	3	55,047–55,341	Prefrontal cortex	–0.73	–0.64	–0.71	0.000084	0.0012	0.00084
Delk1	Doublecortin-like kinase 1	3	55,047–55,341	Nucleus accumbens	–0.80	–0.57	–0.66	0.0000025	0.0055	0.0029
Eif2a	Eukaryotic translation initiation factor 2a	3	58,330–58,361	Prefrontal cortex	–0.72	–0.68	–0.79	0.00012	0.00050	0.000055
Wwtr1	WW domain containing transcription regulator	3	57,260–57,380	Striatum	–0.61	–0.55	–0.75	0.0012	0.0047	0.000063