CRHBP polymorphisms predict chronic pain development following motor vehicle collision

S. D. Linnstaedt
A. V. Bortsov
A. C. Soward
R. Swor
D. A. Peak

See next page for additional authors

Follow this and additional works at: https://academicworks.medicine.hofstra.edu/articles

Part of the Emergency Medicine Commons

Recommended Citation
CRHBP polymorphisms predict chronic pain development following motor vehicle collision

Sarah D. Linnstaedt, PhD1,2,*, Andrey V. Bortsov, MD, PhD1,2,*, April C. Soward, MPH1,2, Robert Swor, MD3, David A. Peak, MD4, Jeffrey Jones, MD5, Niels Rathlev, MD6, David C. Lee, MD7, Robert Domeier, MD8, Phyllis L. Hendry, MD9, and Samuel A. McLean, MD, MPH1,2,10

1TRYUMPH Research Program (SDL, AVB, ACS, SAM)
2Anesthesiology, University of North Carolina, Chapel Hill, NC (SDL, AVB, ACS, SAM)
3Emergency Medicine, William Beaumont Hospital, Royal Oak, Michigan (RS)
4Emergency Medicine, Massachusetts General Hospital, Boston, Massachusetts (DP)
5Emergency Medicine, Spectrum Health System, Grand Rapids, Michigan (JJ)
6Emergency Medicine, Baystate Medical Center, Springfield, Massachusetts (NR)
7Emergency Medicine, North Shore University Hospital, Manhasset, New York (DL)
8Emergency Medicine, Saint Joseph Mercy Health System, Ypsilanti, Michigan (RD)
9Emergency Medicine, University of Florida College of Medicine/ Jacksonville, Florida (PH)
10Emergency Medicine, University of North Carolina, Chapel Hill, NC (SAM)

Abstract

Musculoskeletal pain (MSP) is a common sequela of traumatic stress exposure. While biologic factors contributing to chronic MSP after motor vehicle collision (MVC) have traditionally focused on tissue injury, increasing evidence suggests that neuro/stress/immune processes mediated by stress system activation may play a more dominant role. In a previous study we found that genetic variants in the hypothalamic-pituitary-adrenal (HPA) axis-related gene FKBPS influence vulnerability to persistent MSP 6 weeks after MVC. In the present cohort study (n = 855) we evaluated whether genetic variants in several other important HPA axis-related genes, including the glucocorticoid receptor (NR3C1), corticotropin-releasing hormone receptor R1 (CRHR1), and corticotropin-releasing hormone binding protein (CRHBP), influence risk of chronic MSP over time after MVC. Genetic polymorphism rs7718461 in the CRHBP gene showed significant association (p=0.0012) with overall pain severity during the year after MVC in...
regression models controlling for multiple comparisons. Two additional CRHBP alleles in high Linkage Disequilibrium with rs7718461 also showed trend-level significance. In secondary analyses, a significant interaction between this CRHBP locus (Minor Allele Frequency = 0.33) and time was observed (p = 0.015), with increasing effect observed over time following trauma. A significant CRHBP*FKBP5 interaction was also observed, with substantially increased MSP after MVC in those with a risk allele in both genes compared to either gene alone. The results of this study indicate that genetic variants in two different HPA axis genes predict chronic MSP severity following MVC, and support the hypothesis that the HPA axis is involved in chronic post-MVC MSP pathogenesis.

**Keywords**
Genetic variant; CRHBP; HPA axis; musculoskeletal pain; stress induced hyperalgesia; motor vehicle collision

**INTRODUCTION**

More than 50 million motor vehicle collisions (MVCs) occur worldwide, and more than eleven million Americans experience an MVC each year [1]. The vast majority of individuals experiencing MVC do not have identifiable acute injury, however chronic musculoskeletal pain (MSP) is commonly reported by survivors of such events[25] and results in substantial suffering and diminished health[22; 51].

The pathobiology of chronic MSP development after MVC remains poorly understood. Collision characteristics are poor predictors of MSP outcomes[24; 37]; these data and other lines of evidence indicate that tissue injury is not a dominant factor determining post-MVC MSP outcomes[35]. In contrast, increasing evidence suggests that physiologic systems which shape stress and neuro-immune responses, such as the hypothalamic-pituitary-adrenal (HPA) axis, may play an important role in influencing MSP outcomes after traumatic/stressful events such as MVC[10; 26; 34; 35; 53; 54].

If the HPA axis influences the development of chronic MSP after MVC, then genetic variants affecting key proteins modulating HPA axis function should be associated with vulnerability to chronic post-MVC MSP. In a previous study we found an association between genetic variants in the gene coding for FK506 binding protein 51 (FKBP5), an important regulator of hypothalamic-pituitary-adrenal (HPA) axis function[12], and MSP severity 6 weeks after MVC[10]. In this study we evaluated the association between post-MVC MSP and three other important HPA axis-related genes: the glucocorticoid receptor (NR3C1), corticotropin-releasing hormone (CRH) receptor 1 (CRHR1), and CRH binding protein (CRHBP). In addition, because of increasing evidence that genetic influences on pain outcomes after stress exposure are often time[15] and/or sex[38]-dependent, in secondary analyses we assessed for interactions between significant main effects and sex and time. Also, because of evidence that HPA gene effects may be interactive[16; 45; 46], we explored for interactions between main effects and the previously identified FKBP5 risk allele[10].
METHODS

Study design and population

The details of the MVC study have been reported[44]. In brief, individuals ≥ 18 and ≤ 65 years of age presenting to one of eight Emergency Departments (ED) in four no-fault insurance states within 24 hours of MVC who did not have fracture or other injury requiring hospital admission were enrolled. Patients who were not alert and oriented were excluded, as were pregnant patients, prisoners, patients unable to read and understand English, or patients taking opioids above a total daily dose of 30 mg of oral morphine or equivalent. In addition, because genetic analyses are potentially biased by population stratification, enrollment was limited to non-Hispanic whites (the most common ethnicity at study sites). Informed consent was obtained from all participants and Institutional Review Board (IRB) approval was obtained at all study sites.

DNA collection and genotyping

A blood sample (PAXgene DNA tube) was collected at the time of study enrollment. Following DNA purification (PAXgene blood DNA kit, QIAGEN), genotyping was performed using the Sequenom platform. Sixteen Single Nucleotide Polymorphisms (SNPs) were genotyped across CRHBP, CRHR1, and NR3C1 (Figure 1). These SNPs were selected because they have previously been associated with neuropsychiatric diseases or pain outcomes[23; 28; 31] or because they are tagging SNPs. Two Hapmap samples and two repeat samples were included in each genotyping batch (96 samples) to ensure genotypic accuracy and reliability.

Assessments

Sociodemographic information was collected at the time of the ED visit using a structured interview. Overall pain intensity in the ED and average pain during the month prior to MVC were each assessed by research assistants at the time of the ED interview using a verbal 0–10 numeric rating scale (NRS). Verbal scores have advantages in acute care settings, and verbally administered NRSs have been validated as a substitute for visual analogue scales in acute pain measurement in the ED[5]. Participant follow-up evaluations were conducted six weeks, six months, and one year after MVC using a web-based questionnaire or telephone interview. Each evaluation included an assessment of overall pain intensity in the past week (0–10 NRS); this assessment was used as the primary study outcome measure.

Analyses

Linkage disequilibrium between SNPs in CRHBP, CRHR1, and NR3C1 were explored by calculating Levontin D’ and squared correlation $r^2$ using HaploView[2]. Associations between polymorphisms in CRHBP, CRHR1 and NR3C1 and overall pain intensity in the past week, at 6 weeks, 6 months, and 1 year were assessed using a repeated measures mixed model. Based on the results of previous studies[9; 10; 30; 36], study site, sex, past pain severity, and time from trauma in weeks were included in the model as covariates. Multiple testing was controlled for using the method of principal components[39]. Haplotype frequencies were calculated for the eight possible haplotypes with CRHBP SNPs using the
expectation-maximization algorithm implemented in HaploView and verified using Bayesian estimation of haplotype frequencies implemented in SAS 9.2[29]. As described below, the three SNPs genotyped from the CRHBP genomic region were in high linkage disequilibrium (D’ ≥0.97, Figure 1), and therefore were evaluated as a haplotype. Consistent with previous evidence[46], a dominant model of CRHBP effect provided the best fit to the data and was employed. Interactions between CRHBP haplotypes, sex, time from trauma (in weeks), and FKB5 allele were assessed by including the corresponding product terms in the models. Based on the results of previous studies, FKB5 tagging SNP rs3800373 was used as the FKB5 risk allele[10; 20]. Analyses were performed using SAS 9.2 (SAS Institute Inc, Cary, NC).

RESULTS

Participants
Baseline characteristics of study participants are shown in Table 1. A total of 949 European Americans were enrolled in the study. Most study participants were women less than 40 years of age with some post-secondary education.

Genotyping results
All 16 selected SNPs were successfully genotyped. Genotyped SNPs were in Hardy-Weinberg equilibrium (p > 0.05) and had excellent call rates (≥99%).

Association of HPA-related SNPs with overall MSP 6 weeks, 6 months, and 1 year after MVC
Eight hundred and fifty five of 949 individuals (90%) completed pain assessments at all three timepoints following MVC and were included in the analyses. Results of analyses evaluating the association between HPA axis-related SNPs and MSP outcomes after MVC are shown in Table 2. All three CRHBP SNPs assessed (rs7718461, rs1875999, and rs1053989) were associated with MSP after MVC at the trend-level (p < .01), and one of these SNPs, CRHBP SNP rs7718461, was significantly associated with post-MVC pain burden after adjustment for multiple testing (Table 2). With each of these CRHBP SNPs, the minor allele was associated with increased chronic MSP severity. The FKB5 tagging SNP, rs3800373, which was previously shown to be associated with MSP severity 6 weeks following MVC[10], was also significantly associated with chronic MSP severity across time in the present study, with the minor allele associated with worse MSP outcomes (Table 2).

Secondary analyses
Evaluation for potential gene-time interactions—Because all three CRHBP SNPs were in very high Linkage Disequilibrium (LD), and because the minor allele of each SNP was associated with pain vulnerability, subsequent analyses were performed with the three CRHBP SNPs grouped into a haplotype. Of eight possible haplotypes constituted from the three SNPs, only H1, H2, and H3 had frequencies above 0.01 (Supplementary Table 1). H2 contained all three CRHBP risk alleles (G-G-T, frequency = 0.33); in a repeated measures mixed model, H2 haplotype was significantly associated with post-MVC pain severity after

Pain. Author manuscript; available in PMC 2017 January 01.
adjustment for assessment week and study site (F(1,900)=7.7, p=0.006). Therefore the H2 haplotype was utilized in subsequent analyses.

Because of evidence that genetic influences on pain outcomes after stress exposure are often time-dependent, we assessed for time-dependent differences in the influence of CRHBP H2 haplotype on post-MVC MSP severity, and in the influence of FKB5 allele rs3800373 on post-MVC MSP severity. A significant time-CRHBP H2 haplotype effect was observed (F(1,897)=5.9, p=0.015, Table 3, Figure 2 Panel A), with the presence of a CRHBP H2 haplotype minor allele having an increasing effect with increasing time from trauma. No evidence for an interaction was observed between FKB5 allele rs3800373 and time (F(1,897)=0.28, p=0.40, Table 3). These results are also presented in graphical format in Panel A of Figure 2.

Evaluation for potential gene-sex interactions—As described above, because of evidence that genetic influences on pain outcomes after stress exposure are often sex-dependent, we also evaluated for an interaction between sex and H2 haplotype and chronic post-MVC MSP severity. CRHBP analyses were stratified by timepoint because of the above evidence that CRHBP effects are time-dependent. No interaction was observed (F(1,843)=0.21, p=0.65 at 6 weeks; F(1,822)=1.73, p=0.19 at 6 months; F(1,842)=0.76, p=0.38 at 1 year). Similarly, no interaction was observed between sex and FKB5 allele rs3800373 and pain over time after MVC (F(1,843)=0.66, p=0.42).

Evaluation for potential gene-gene interactions—In addition, because of previous evidence that HPA gene effects may be interactive, we tested for an interaction between the FKB5 genotype rs3800373 and CRHBP H2 haplotype. CRHBP analyses were stratified by timepoint because of the above evidence that CRHBP effects are time-dependent. A highly significant interaction was observed between FKB5 and CRHBP risk allele and MSP pain outcome 6 weeks after MVC (p <0.0001, Table 4). For example, individuals with two copies of the FKB5 risk allele and one or more copies of the CRHBP risk allele had MSP nearly double the severity of individuals without the additional CRHBP risk allele(s) (Table 4, Figure 2 Panel B). This interaction did not reach statistical significance at later timepoints, although a similar trend in effect size was observed at the 6 month timepoint.

DISCUSSION

Currently little is known about the biological pathways involved in chronic pain development following trauma exposure or which individuals are at high risk of developing MSP following exposures such as MVC. This study is the second study to identify a genetic association between HPA axis-related genes and MSP outcomes after MVC. In our prior study using the same dataset, we reported that the same FKB5 risk alleles predict MSP severity six weeks after both MVC and sexual assault. (That sexual assault study did not include data from the later follow-up timepoints used in the present analysis.) In the present study, haplotypes within another important HPA axis gene, CRHBP, predicted MSP burden during the year after MVC, with the adverse effect of a vulnerability allele increasing over time. The effect of CRHBP also interacted with FKB5 vulnerability alleles identified in the
previous study, such that individuals with vulnerability alleles at both of these HPA axis genes had substantially greater MSP after MVC than individuals with a vulnerability allele at only one gene. This finding is consistent with the results of a previous study that found an interaction between CRHBP and FKBP5 genes and mental health outcomes following childhood trauma[46].

CRH-BP and FK506 binding protein 51 (FKBP5) are key regulatory proteins affecting HPA axis function. Neural input into the hypothalamus causes the release of Corticotropin-Releasing Hormone (CRH), which initiates activation of the HPA axis. CRH-BP has complex effects[49; 60], evidence from animal studies suggests that increased CRH-BP expression inhibits HPA axis activation[48]. HPA axis activation results in the release of cortisol which diffuses through the cell membrane of cells in the body and binds to the glucocorticoid receptor (GR)[62]. FKBP5 regulates the sensitivity of the glucocorticoid receptor (GR) to cortisol[6]. Higher expression of FKBP5 has been shown to reduce cortisol binding affinity of the GR [14] and nuclear translocation of the GR[61] (i.e., result in GR resistance to cortisol), with accompanying increased plasma cortisol levels[14; 47].

Available evidence indicates that FKBP5 variants associated with worsened chronic MSP outcomes following MVC in the present study cause GR resistance to cortisol [7; 10; 14; 47]. In contrast, the physiologic effect of CRH-BP variants associated with worsened chronic pain outcomes following MVC in the present study are not well understood. To date, no studies have identified functional effects of variants in the CRHBP locus[8]. Further studies are needed to understand the functional effects of the identified CRHBP variants.

Epistatic interactions such as the interaction observed in the present study between the FKBP5 and CRHBP risk alleles are common[42]. This interaction was observed 6 weeks after MVC but not at 6 months or 1 year. The time-dependent nature of these interactions could be due to decreased power at later timepoints, as more individuals recover and have reduced MSP scores. This hypothesis is supported by the fact that the same direction of effect was observed at the 6 month and 1 year timepoints, but the effect weaker. Another potential contributor to the different effect of these interactions over time is that stress-related biologic effects are often markedly time-dependent[15].

The results of this study cannot determine to what extent the HPA axis is directly involved in the pathogenesis of post-MVC MSP or is a marker for pathobiologic processes mediated by other physiologic systems. Several lines of evidence from other studies suggest however that the HPA axis may itself have a direct contributing role. First, in a well-elucidated animal model of stress-induced hyperalgesia, widespread hyperalgesia was demonstrated to be due to the direct effect of persistently elevated levels of glucocorticoids and catecholamines on primary sensory afferents[27]. In addition, glucocorticoid systems exert an important influence on immune system function, and CRHBP and FKBP5 risk haplotypes may influence post-stress outcomes in part via mechanisms which lead to the increased production of pro-inflammatory mediators (e.g. cytokines)[17; 50]. Such mediators may promote allodynia and hyperalgesia both by sensitizing peripheral and central afferents directly[18; 19; 32] and by sensitizing CNS neurons via an afferent feedback mechanism[18; 19; 32; 56–58]. CRHBP and FKBP5 risk haplotypes may also affect sensory processing after
stress exposure by their effect on central nervous system glucocorticoid pathways (e.g. [52; 55]).

Findings regarding the CRHBP allele evaluated in the present study are consistent with data linking this locus with other stress-related neuropsychiatric disorders. CRHBP rs1875999, one of the three CRHBP variants comprising the CRHBP H2 haplotype in the present study and the only one of these SNPs conserved across multiple mammalian species (http://genome.ucsc.edu/ Dec. 2013 (GRCh38/hg38 Assembly), has been associated with vulnerability to attempting suicide after stress exposure[13; 46] and to stress-related drug addiction[28]. CRHBP rs1875999 has also been associated with an increased number of pain sites[23]. CRHBP rs1875999 is located in the 3’UTR of the CRHBP gene; this gene region functions as a post-transcriptional regulatory region by determining the localization, stability, and translational efficiency of a gene transcript[3]. Molecular factors currently known to influence protein translation via the 3’UTR include binding by regulatory proteins or by microRNA[4; 43]. Examination of the genomic region surrounding the two CRHBP SNPs in the 3’UTR identified in this study, do not suggest direct influence of the allele on RNA binding protein binding or microRNA binding. However, the Adenylate-uridylate (AU) content of the CRHBP 3’UTR is 66 percent (average 3’UTR AU content ~55 percent [41]) and there are a number of AU-rich elements (AREs) in the CRHBP 3’UTR[21]. Therefore one potential molecular effect mediating the association between CRHBP H2 haplotype and chronic post-MVC pain outcomes is that this polymorphism affects CRHBP protein levels via modulating regulatory protein binding to ARE regions. Indeed, one such regulatory protein, HuD (ELAVL4), has previously been shown to affect neuronal plasticity[40]. Further studies are needed to evaluate this hypothesis.

The reasons for a lack of association between NR3C1 and CRHR1 genetic variants and chronic MSP are not known. Given the relatively low MAFs for many of the NR3C1 and CRHR1 alleles evaluated in comparison to the evaluated CRHBP alleles, it is possible that our sample size was underpowered for these alleles. In addition, previous associations between NR3C1 variants and post-trauma outcomes have suggested that functional variants in this gene affect hypermethylation of the promoter region[11; 33; 59]. Because the genetic variants assessed in our study were mostly intronic and downstream of the promoter, it is possible that we did not tag alleles responsible for methylation status of NR3C1. If this is the case, then lack of adjustment for this important covariate (hypermethylation status) may have reduced our power or biased our findings towards the null.

Several limitations should be noted when interpreting the results of this study. First, to avoid confounding by population stratification our study only enrolled European Americans. The generalizability of our findings to other race/ethnic groups is unknown. Second, results regarding the association between the CRHBP allele and post-MVC MSP outcome identified in the present study have not been replicated in a second cohort. However, this is not a novel finding of a previously unstudied gene locus. Instead this is a finding of an association between a stress-related outcome (chronic post-MVC MSP) and a gene locus previously associated with other stress-related disorders, in a biologic pathway that has been implicated in the pathogenesis of chronic MSP development in both animal and human studies. Thus from a Bayesian viewpoint, we believe that the post-test probability of our

Pain. Author manuscript; available in PMC 2017 January 01.
allele actually contributing to risk of chronic post-MVC MSP exceeds that of a novel replicated allele, and that our finding is likely to be a true positive. Finally, another limitation is that we enrolled only about half of the potentially eligible participants. The generalizability of our results among individuals who declined enrollment is not known. These limitations are consistent with other studies enrolling subjects after an acute aftermath of trauma in an ethical manner.

CONCLUSIONS

The results of this study suggest that genetic variants in corticotropin-releasing hormone binding protein, a gene affecting glucocorticoid signal transduction, influence the severity of pain symptoms experienced one year after MVC trauma. These findings add further evidence that physiologic systems which shape stress and neuroimmune responses, such as the HPA axis, may play an important role in influencing MSP outcomes after traumatic/stressful events such as MVC. These findings also suggest that glucocorticoid pathways might be a good target of interventions aimed at improving recovery and diminishing chronic pain after trauma exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank the participants for taking part in this study.

The project described was supported by Award Number R01AR056328 from the National Institute of Arthritis and Musculoskeletal and Skin diseases.

References


34. McLean SA. The Potential Contribution of Stress Systems to the Transition to Chronic WAD. Spine. 2011


53. Ulirsch JC, Weaver MA, Bortsov AV, Soward AC, Swor RA, Peak DA, Jones JS, Rathlev NK, Lee DC, Domeier RM, Hendry PL, McLean SA. No man is an island: Living in a disadvantaged neighborhood influences chronic pain development after motor vehicle collision, and this effect is moderated by common genetic variation influencing HPA axis function. Pain. 2014


Pain. Author manuscript; available in PMC 2017 January 01.
61. Wochnik GM, Ruegg J, Abel GA, Schmidt U, Holsboer F, Rein T. FK506-binding proteins 51 and
52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid
[PubMed: 15591061]

62. Zhou J, Cidlowski JA. The human glucocorticoid receptor: one gene, multiple proteins and diverse
Figure 1.
Genomic location and Linkage Disequilibrium Plots of CRHBP, CRHR1, and NR3C1 polymorphisms analyzed in this study. Color and numbers represent D’ values (dark red=high inter-SNP D’; white=low inter-SNP D’).
Figure 2.
Trajectories of pain severity by genotype. In panel A, patients are grouped by their CRHBP or FKBP5 genotype. CRHBP H2 (+) denotes the presence of at least one H2 risk haplotype; FKBP5 rs3800373 (+) denotes presence of the risk allele. In panel B, patients are grouped by the combination of their CRHBP and FKBP5 genotypes. CRHBP H2 (+) denotes the presence of at least one H2 risk haplotype; FKBP5 rs3800373 (+) denotes the presence of two risk alleles.
Table 1

Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled, n</td>
<td>949</td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>36(13)</td>
</tr>
<tr>
<td>Females, n (%</td>
<td>575(61)</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
</tr>
<tr>
<td>8–11 yrs</td>
<td>42(4)</td>
</tr>
<tr>
<td>HS</td>
<td>184(19)</td>
</tr>
<tr>
<td>Post-HS</td>
<td>57(6)</td>
</tr>
<tr>
<td>Some college</td>
<td>311(33)</td>
</tr>
<tr>
<td>College</td>
<td>237(25)</td>
</tr>
<tr>
<td>Post-college</td>
<td>113(12)</td>
</tr>
<tr>
<td>Overall pain, 0–10 NRS, mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Past month</td>
<td>0.4(0.5)</td>
</tr>
<tr>
<td>ED</td>
<td>5.5(2.4)</td>
</tr>
</tbody>
</table>
Table 2

Association of the Hypothalamic-Pituitary-Adrenal axis alleles with chronic overall pain severity

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Alleles</th>
<th>MAF</th>
<th>Homozygous Major</th>
<th>Heterozygous</th>
<th>Homozygous Minor</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs258763</td>
<td>NR3C1</td>
<td>T/A</td>
<td>0.43</td>
<td>3.5 (3.2, 3.8)</td>
<td>3.4 (3.2, 3.7)</td>
<td>3.5 (3.1, 3.8)</td>
<td>0.83</td>
</tr>
<tr>
<td>rs9324916</td>
<td>NR3C1</td>
<td>C/G</td>
<td>0.23</td>
<td>3.4 (3.2, 3.6)</td>
<td>3.4 (3.2, 3.7)</td>
<td>4.1 (3.4, 4.7)</td>
<td>0.57</td>
</tr>
<tr>
<td>rs2963155</td>
<td>NR3C1</td>
<td>A/G</td>
<td>0.21</td>
<td>3.4 (3.2, 3.6)</td>
<td>3.5 (3.3, 3.8)</td>
<td>3.5 (2.8, 4.2)</td>
<td>0.46</td>
</tr>
<tr>
<td>rs9324918</td>
<td>NR3C1</td>
<td>A/G</td>
<td>0.14</td>
<td>3.5 (3.3, 3.6)</td>
<td>3.5 (3.2, 3.9)</td>
<td>3.1 (2.1, 4.0)</td>
<td>0.81</td>
</tr>
<tr>
<td>rs9324924</td>
<td>NR3C1</td>
<td>G/T</td>
<td>0.33</td>
<td>3.4 (3.2, 3.6)</td>
<td>3.5 (3.3, 3.8)</td>
<td>3.5 (3.0, 3.9)</td>
<td>0.47</td>
</tr>
<tr>
<td>rs12942300</td>
<td>CRHR1</td>
<td>T/A</td>
<td>0.15</td>
<td>3.5 (3.3, 3.7)</td>
<td>3.3 (3.0, 3.6)</td>
<td>3.3 (2.4, 4.2)</td>
<td>0.18</td>
</tr>
<tr>
<td>rs7209436</td>
<td>CRHR1</td>
<td>C/T</td>
<td>0.43</td>
<td>3.3 (3.0, 3.6)</td>
<td>3.6 (3.3, 3.8)</td>
<td>3.5 (3.1, 3.8)</td>
<td>0.16</td>
</tr>
<tr>
<td>rs4792887</td>
<td>CRHR1</td>
<td>C/T</td>
<td>0.09</td>
<td>3.5 (3.3, 3.7)</td>
<td>3.4 (3.0, 3.8)</td>
<td>3.9 (2.2, 5.6)</td>
<td>0.77</td>
</tr>
<tr>
<td>rs17689378</td>
<td>CRHR1</td>
<td>C/T</td>
<td>0.23</td>
<td>3.4 (3.2, 3.6)</td>
<td>3.5 (3.2, 3.8)</td>
<td>3.5 (2.8, 4.1)</td>
<td>0.67</td>
</tr>
<tr>
<td>rs12936511</td>
<td>CRHR1</td>
<td>C/T</td>
<td>0.03</td>
<td>3.5 (3.3, 3.6)</td>
<td>3.5 (2.9, 4.2)</td>
<td>3.5 (0.9, 6.2)</td>
<td>0.81</td>
</tr>
<tr>
<td>rs81189</td>
<td>CRHR1</td>
<td>G/C</td>
<td>0.48</td>
<td>3.3 (3.0, 3.6)</td>
<td>3.6 (3.4, 3.8)</td>
<td>3.4 (3.1, 3.7)</td>
<td>0.18</td>
</tr>
<tr>
<td>rs16940665</td>
<td>CRHR1</td>
<td>T/C</td>
<td>0.23</td>
<td>3.4 (3.2, 3.6)</td>
<td>3.5 (3.3, 3.8)</td>
<td>3.5 (2.8, 4.1)</td>
<td>0.62</td>
</tr>
<tr>
<td>rs16940674</td>
<td>CRHR1</td>
<td>C/T</td>
<td>0.23</td>
<td>3.4 (3.2, 3.6)</td>
<td>3.5 (3.2, 3.8)</td>
<td>3.5 (2.9, 4.2)</td>
<td>0.64</td>
</tr>
<tr>
<td>rs7718461</td>
<td>CRHBP</td>
<td>A/G</td>
<td>0.38</td>
<td>3.1 (2.9, 3.3)</td>
<td>3.5 (2.2, 3.7)</td>
<td>3.5 (3.4, 3.7)</td>
<td>0.0012&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs1875999</td>
<td>CRHBP</td>
<td>A/G</td>
<td>0.34</td>
<td>3.2 (3.0, 3.4)</td>
<td>3.5 (3.4, 3.7)</td>
<td>3.4 (3.1, 3.7)</td>
<td>0.0061</td>
</tr>
<tr>
<td>rs1053989</td>
<td>CRHBP</td>
<td>G/T</td>
<td>0.37</td>
<td>3.1 (3.0, 3.3)</td>
<td>3.5 (3.4, 3.7)</td>
<td>3.4 (3.1, 3.7)</td>
<td>0.0054</td>
</tr>
<tr>
<td>rs3800373</td>
<td>FKBP5</td>
<td>T/G</td>
<td>0.30</td>
<td>3.1 (2.9, 3.3)</td>
<td>3.7 (3.4, 3.9)</td>
<td>3.5 (2.9, 4.0)</td>
<td>0.0004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Repeated measures regression model (6 weeks, 6 month, 1 year), dominant genetic model, adjusted for week, past pain severity, sex, study site.

<sup>b</sup> p values are significant after controlling for multiple testing using the method of principal components (the number of effective tests is 12, adjusted significance threshold is 0.05/12 = 0.0042)
Interaction between time after motor vehicle collision and effect of *CRHBP* and *FKBP5* risk allele on musculoskeletal pain severity.

<table>
<thead>
<tr>
<th>Gene/SNP</th>
<th>Risk allele</th>
<th>Pain Intensity (0–10 numeric rating scale)</th>
<th>Interaction p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 weeks</td>
<td>6 months</td>
</tr>
<tr>
<td>CRHBP H2</td>
<td>Present</td>
<td>3.9 (3.7, 4.2)</td>
<td>3.3 (3.0, 3.5)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>3.7 (3.4, 4.0)</td>
<td>2.8 (2.6, 3.1)</td>
</tr>
<tr>
<td>FKBP5 rs3800373</td>
<td>Present</td>
<td>4.1 (3.9, 4.4)</td>
<td>3.4 (3.2, 3.7)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>3.6 (3.3, 3.9)</td>
<td>2.9 (2.6, 3.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Repeated measures regression model (6 weeks, 6 month, 1 year), dominant genetic model with a gene-week interaction term, adjusted for week, past pain severity, sex, study site

<sup>b</sup>*CRHBP* H2 refers to haplotype 2 of the gene encoding corticotropin-releasing hormone binding protein and *FKBP5* is the gene encoding FK506 binding protein.
### Table 4

*CRHBP × FKBP5* interaction predicting overall pain intensity (0–10 NRS) over time following motor vehicle collision

<table>
<thead>
<tr>
<th>Time after motor vehicle collision</th>
<th>FKBP5 rs3800373 genotype</th>
<th>Number of CRHBP H2 haplotypes</th>
<th>Mean pain severity (95% CI)</th>
<th>p-value</th>
<th>Gene-gene interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six weeks</td>
<td></td>
<td>1 or 2</td>
<td>3.8 (3.6, 4.1)</td>
<td>0.844</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>3.9 (3.6, 4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1 or 2</td>
<td>5.5 (4.6, 6.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>2.8 (1.8, 3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Six months</td>
<td></td>
<td>1 or 2</td>
<td>3.2 (3.0, 3.5)</td>
<td>0.1514</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>3.0 (2.6, 3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1 or 2</td>
<td>4.4 (3.4, 5.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>3.0 (1.8, 4.2)</td>
<td>0.0596</td>
<td></td>
</tr>
<tr>
<td>One year</td>
<td></td>
<td>1 or 2</td>
<td>3.6 (3.3, 3.8)</td>
<td>0.0038</td>
<td>0.5774</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>3.0 (2.7, 3.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1 or 2</td>
<td>3.9 (2.8, 4.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>3.2 (1.9, 4.4)</td>
<td>0.3326</td>
<td></td>
</tr>
</tbody>
</table>

*Genotype* is coded 0=homozygous on major allele or heterozygous, 1=homozygous on minor allele. Gene × gene × time 3 way interaction, p = 0.0031.