Published in final edited form as:

Neuromolecular Med. 2014 March ; 16(1): 83–93. doi:10.1007/s12017-013-8255-9.

Complex multilocus effects of catechol-O-methyltransferase haplotypes predict pain and pain interference 6 weeks after motor vehicle collision

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Abstract

Catechol-*O*-methyltransferase, encoded by *COMT* gene, is the primary enzyme that metabolizes catecholamines. *COMT* haplotypes have been associated with vulnerability to persistent non-traumatic pain. In this prospective observational study, we investigated the influence of *COMT* on persistent pain and pain interference with life functions after motor vehicle collision (MVC) in 859 European American adults for whom overall pain (0–10 numeric rating scale) and pain interference (Brief Pain Inventory) were assessed at week 6 after MVC. Ten single nucleotide polymorphisms (SNPs) spanning the *COMT* gene were successfully genotyped, nine were present in three haploblocks: block 1 (rs2020917, rs737865, rs1544325), block 2 (rs4633, rs4818, rs4680, rs165774) and block 3 (rs174697, rs165599). After adjustment for multiple comparisons, haplotype TCG from block 1 predicted decreased pain interference ($p = .004$). The pain-protective effect of the low pain sensitivity (LPS, CGGG) haplotype from block 2 was only observed if at least one TCG haplotype was present in block 1 (haplotype $\times$ haplotype interaction $p = .002$ and $< .0001$ for pain and pain interference, respectively). Haplotype AG from block 3 was associated with pain and interference in males only (sex $\times$ haplotype interaction $p = .005$ and $.0005$, respectively). These results suggest that genetic variants in the distal promoter are important contributors to the development of persistent pain after MVC, directly and via the interaction with haplotypes in the coding region of the gene.

Keywords

Catechol-*O*-methyltransferase; musculoskeletal pain; motor vehicle collision; stress

INTRODUCTION

COMT has been shown to influence an array of complex phenotypes, including pain phenotypes (Nackley et al. 2006b; Diatchenko et al. 2005; Meyer-Lindenberg et al. 2006; Zubieta et al. 2003; Smolka et al. 2007; Reuter and Hennig 2005). Initially it was widely assumed that variations in *COMT* activity in humans were caused only by the non-

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CONFLICT OF INTEREST

Dr. Diatchenko is a co-founder and equity stock holder in Algynomics, Inc. Other authors declare no conflict of interest.

synonymous valine (Val) to methionine (Met) substitution at codon 158 (rs4680), which results in reduced thermostability and reduced activity of the enzyme (Lotta et al. 1995). Numerous studies identified associations between the low-activity Met158 allele and several pain phenotypes (e.g. (Smith et al. 2011; Cohen et al. 2009; Barbosa et al. 2012; Jacobsen et al. 2012; van Meurs et al. 2009; Gursoy et al. 2003)), however the observed associations were often modest and occasionally inconsistent (Tander et al. 2008; Hocking et al. 2010; Nicholl et al. 2010), suggesting that additional SNPs in the *COMT* gene modulate *COMT* activity.

In a study examining the association between *COMT* genotypes in the central haploblock of the *COMT* gene and variability in human pain perception, the Val158Met polymorphism alone was not significantly associated with pain sensitivity (Diatchenko et al. 2005). Instead, three common haplotypes in the central haploblock, including two synonymous SNPs (rs4633 and 4818) and the nonsynonymous Val158Met SNP, were found to code for different levels of enzymatic activity and influence vulnerability to persistent musculoskeletal pain (Diatchenko et al. 2005). These differences in *COMT* enzyme activity were subsequently demonstrated to be mediated by haplotype-dependent differences in secondary RNA structure (Nackley et al. 2006a). The robust effect of these haplotype-dependent differences in secondary RNA structure on *COMT* enzyme activity was found to be much greater than the modest effect of the Val158Met substitution alone (Nackley et al. 2006a).

Despite these data, studies examining the association between *COMT* haplotypes in the central haploblock of the *COMT* gene and persistent musculoskeletal pain outcomes have produced inconsistent results. While *COMT* haplotypes predicted fibromyalgia symptom severity in Spanish (but not Mexican) women (Vargas-Alarcon et al. 2007), two other population-based studies failed to demonstrate an association of *COMT* haplotypes and chronic widespread pain (Hocking et al. 2010; Nicholl et al. 2010). One potential contributor to these inconsistent results may be that important interactions exist between these haplotypes and other variants in *COMT*. This hypothesis is supported by evidence that *COMT* variants outside the central haploblock, including variants in the distal promoter (P2) region and 3'-untranslated region, influence complex phenotypes (Meyer-Lindenberg et al. 2006; Shifman et al. 2002; Gaysina et al. 2013; Shibata et al. 2009). Another potential contributor to inconsistent findings in previous association studies evaluating haplotypes in the central *COMT* haploblock may be sex-specific effects. Evidence suggests that important differences in the influence of *COMT* genotypes may exist between men and women (reviewed in (Tunbridge and Harrison 2011)).

In this prospective study we evaluated the influence of haplotypes from three functionally important *COMT* loci (promoter P2, coding region, and 3'-untranslated region) on vulnerability to persistent pain 6 weeks after motor vehicle collision (MVC). We hypothesized that *COMT* haplotypes, and/or interactions between haplotypes from these three loci and patient sex, would influence pain severity six weeks after experiencing an MVC. In addition, because pain-related interference with global life function is an important health outcome in patients with pain (Dworkin et al. 2005) and because *COMT* may influence not only pain severity but also psychological processes that influence function in the presence of pain (George et al. 2008b; George et al. 2008a), we also evaluated the influence of these haplotypes on pain-related interference six weeks after MVC.

MATERIALS AND METHODS

Participants

The details of the CRASH study have been reported (Platts-Mills et al. 2011). In brief, individuals 18 and 65 years of age presenting to one of eight emergency departments 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, patients taking a β -adrenoreceptor antagonist, or patients taking opioids above a total daily dose of 30 mg of oral morphine or equivalent. Participant race was determined via self-report. Enrollment was limited to non-Hispanic whites (the most common ethnicity at study sites) because the study included the collection of genetic data and genetic analyses are potentially biased by population stratification (Diatchenko et al. 2007). Informed consent was obtained from all participants and IRB approval was obtained at all study sites.

DNA collection and genotyping

Blood (8.5cc) was collected using PAXgene DNA storage tubes. Blood samples were then refrigerated at the study site and shipped in batches every 2 weeks to Beckman Coulter Genomics, Inc, Morrisville, NC. DNA purification was performed using PAXgene™ blood DNA kit (Qiagen, Valencia, CA). Average DNA yield was 275 μ g per sample. Genotyping was performed at batches using the Sequenom platform (Sequenom, Inc., San Diego, CA). Two Hapmap samples and 2 repeat samples were included in each genotyping batch (96 samples) to ensure genotypic accuracy and reliability. Repeated genotyping demonstrated greater than 98% call agreement.

Outcome assessments

Overall pain intensity six weeks after MVC (defined in this study as “persistent pain”) was assessed via telephone interview or web-based questionnaire using a verbal 0–10 numeric rating scale (NRS) assessing average overall pain intensity during the past week. Verbally administered NRSs have been validated as a substitute for Visual Analog Scales in acute pain measurement in the emergency department (Bijur et al. 2003).

Pain interference with life functions was assessed using the Brief Pain Inventory (BPI) (Cleeland and Ryan 1994; Keller et al. 2004). Interference with each life function (general activity, walking ability, mood, relations with other people, sleep and enjoyment of life) was assessed on a scale from 0 to 10, where 0 represents “does not interfere” and 10 indicates “interferes completely”. Interference total score was calculated by adding up interference subscale values for each individual.

Analyses

All genotyped SNPs were tested for Hardy-Weinberg equilibrium (HWE) using a chi-square test. SNPs that were not in HWE, SNPs with call rates below 85%, and SNPs with minor allele frequency less than 0.05 were excluded from analyses.

Linkage disequilibrium between *COMT* SNPs was explored by calculating Levontin D' and squared correlation r^2 using HaploView (Barrett et al. 2005). Haploblocks were estimated using the method of confidence intervals (Gabriel et al. 2002). *COMT* haplotypes and their population frequencies were estimated using the expectation-maximization algorithm implemented in HaploView and were then verified using Bayesian estimation of haplotype frequencies implemented in HAPLOTYPE procedure (SAS version 9.2, SAS Institute, Cary, NC) (Lin et al. 2002).

The associations between *COMT* haplotypes and pain outcomes in the CRASH cohort were assessed using general linear models. Study site was included as a covariate in these models due to potential genetic heterogeneity between study recruitment centers. Interaction between haplotypes and interaction between haplotypes and patient sex were assessed by introducing the corresponding product terms into the models. Partial F-test was used to evaluate whether the removal of the non-significant terms affected model fit. Least square means were output from the model and post-hoc pairwise comparisons of means were performed. Bonferroni correction of the significance level was performed by dividing 0.05 by the number of estimated haplotypes.

RESULTS

Study population

A total of 10,629 individuals who presented to the emergency department for evaluation in the hours after MVC were screened, 1,416 were eligible, 969 consented to study participation, and 948 completed baseline evaluation. The recruitment flowchart, with major reasons for ineligibility and refusal, is shown in Supplementary Figure 1. Nine hundred and forty five individuals were successfully genotyped, 6 week follow-up evaluation was obtained in 859/945 (91%). Participants who were lost to follow-up were more likely to be younger, less-educated males; they did not, however, were different from the rest of the cohort in terms of baseline pain severity (Table 1).

Genotyping results and haplotype estimation

Ten SNPs spanning the *COMT* gene were genotyped. Call rates were excellent (99.9%) for nine SNPs and good for rs165599 (96.4%). All SNPs were in Hardy-Weinberg equilibrium (Chi-square $p > .05$, Supplementary Table 1). Using data from all 945 genotyped DNA samples, three haploblocks were identified by the method of confidence intervals (Gabriel et al. 2002) (Figure 1, A). Haploblocks 1, 2, and 3 included three, four, and three common haplotypes, respectively (Figure 1, B). Haplotypes in block 1 were denoted as A1–A3; haplotypes in block 3 were denoted as B1–B3. Haplotypes in block 2 corresponded to those identified in a previous study (Diatchenko et al. 2005) and included the low pain sensitivity (LPS) haplotype, the high pain sensitivity (HPS) haplotype, and two variants of the average pain sensitivity (APS1 and APS2) haplotype (Table 2).

Haplotype frequencies were estimated using the Expectation-Maximization algorithm; verification using Bayesian estimation yielded virtually identical results (data not shown). Within each block, the above described common haplotypes accounted for approximately 99% of haplotypic diversity. Haplotype pair assignment probability for blocks 1, 2, and 3 was greater than .99 in 943, 941, and 911 individuals, respectively. Because haplotype uncertainty in a small proportion of individuals (<5% of the total sample) is unlikely to substantially bias study results (Kraft and Stram 2007), for further analyses these individuals were assigned to the most probable haplotype pair.

Bivariate analyses evaluating associations between *COMT* haplotype and persistent pain and pain interference 6 weeks after MVC

Under the genotypic model adjusted for study site, LPS haplotype was significantly protective against persistent pain at the nominal level $\alpha = 0.05$ (Table 2). Three haplotypes were associated with increased pain interference at the nominal level $\alpha = 0.05$: LPS, haplotype A3, and haplotype B2. After applying Bonferroni correction ($\alpha = 0.05/10 = 0.005$), only A3 remained a significant ($p = 0.004$) predictor of pain interference. Individuals heterozygous at the A3 allele had the highest pain interference,

those homozygous had the lowest pain interference, and individuals without a haplotype A3 had an intermediate phenotype.

Multivariate analyses including haplotype × haplotype and haplotype × sex interactions

Multivariate regression analyses with interaction terms were used to evaluate the potential influence of haplotype × haplotype and haplotype × sex interactions on pain and pain-related interference outcomes. Haplotypes associated at the $p < 0.05$ level with pain and pain-related interference outcomes in bivariate analyses (A3, LPS, and B2), sex, study site, and all possible two-way interactions were included as model terms. Interactions A3×LPS and B2×Sex were highly significant in both models (Table 3). Removal of non-significant and borderline-significant interaction terms did not affect the overall fit of the models (partial F-test $p > 0.1$), and both interactions (A3×LPS and B2×Sex) remained highly significant (Table 3).

Subgroup analyses of haplotypexhaplotype and haplotypexsex interactions

To better understand the identified A3×LPS interaction, the influence of an LPS allele on pain-related outcomes was compared among individuals in the MVC cohort with (410, 48%) and without (445, 52%) at least one A3 allele. Among those with one or more copies of the A3 allele, LPS haplotype had a nearly linear protective effect on pain-related outcomes, reducing overall pain severity by 0.8 ± 0.3 units ($p = 0.002$, Figure 2, A) and pain interference by 5.7 ± 1.6 units ($p = 0.0004$, Figure 2, B) for each LPS allele copy present. In contrast, among individuals without at least one A3 allele, LPS copy number had no effect on pain or pain interference (Figure 2).

To evaluate the B2×sex interaction, the effect of B2 haplotype on pain-related outcomes after MVC was compared among men (321, 37%) and women (538, 63%). Because of the low frequency of haplotype B2 (5.4%), heterozygous ($n = 83$) and B2/B2 homozygous ($n = 5$) individuals were combined into one category. Among men, the presence of one or two copies of haplotype B2 was associated with increased overall pain (by 1.5 ± 0.5 scale units, $p = 0.005$, Figure 3, A) and increased pain interference (by 12 ± 4 scale units, $p = 0.0005$, Figure 3, B). In contrast, among women the presence of one or two copies of haplotype B2 had no effect on pain and pain interference (Figure 3).

To explore whether this sex difference in B2 haplotype influence might be limited to premenopausal women, we repeated the analysis among only men and women who were age 55 or older at the time of the MVC ($n = 108$). Although the interaction between B2 haplotype and sex in this much smaller sample was not statistically significant ($p = 0.25$ for pain interference and $p = 0.093$ for pain), effect sizes were similar (data not shown). These data suggest that the interaction between B2 haplotype and sex may not be limited to premenopausal women alone.

DISCUSSION

In this prospective cohort study of adult European Americans presenting to the emergency department after MVC, *COMT* haplotype×haplotype and haplotype×sex interactions were much stronger predictors of pain outcomes 6 weeks after MVC than individual haplotypes. In the overall cohort, the LPS haplotype in the *COMT* coding region was nominally associated with pain and interference, and haplotype A3 in the P2 promoter region predicted pain interference with a significant overdominant effect. However, as evident from the F-values in Tables 2 and 3, the interaction of the A3 and LPS haplotypes was much more strongly associated with pain and pain interference outcomes after MVC than either haplotype alone. (The fact that the LPS haplotype was largely dependent on distal P2

promoter genotype suggests that the *COMT* P2 locus is also functional.) Similarly, the effect of haplotype B2 in the 3'-untranslated region was associated with increased overall pain and pain interference after MVC in men, but not in women.

The present finding that *COMT* distal P2 promoter genotype influences pain outcomes after MVC is consistent with current understanding of *COMT* isoform expression and physiology. The P2 promoter influences MB-*COMT* expression, and MB-*COMT* is the predominant form of the enzyme in the central nervous system (CNS) (Chen et al. 2004). Thus genetic variants in P2 are most likely to affect the function of important pain pathways in the CNS. In addition, the MB-*COMT* isoform also has a higher affinity to catecholamines at the physiologic concentrations of catecholamines found in the brain (Roth 1992).

Our findings of the interaction between genotypes in the coding region of *COMT* and genotypes in the P2 promoter region are in accordance with several previous studies. In a study examining the influence of *COMT* polymorphisms on brain function, two SNPs in the P2 region, rs737865 and rs2075507, when analyzed together with the Val158Met polymorphism, were shown to critically affect prefrontal cortex function (Meyer-Lindenberg et al. 2006). Of note, allele C at rs737865 is a tagging allele for haplotype A3 in our study (Figure 1, B) and is in high linkage disequilibrium with *HindIII*, a potentially functional deletion in the P2 promoter (Palmatier et al. 2004). In another study, allele C at rs737865 was found to be associated with reduced *COMT* transcription in the central nervous system (Bray et al. 2003). Finally, interactions between P2 promoter region genetic variants and the coding region variants have been associated with vulnerability to schizophrenia (Shifman et al. 2002).

One of the hypothetical mechanistic explanations of the interaction between promoter P2 variants and coding region haplotypes employs a potentially non-linear relationship between *COMT* activity and vulnerability to persistent pain (Figure 4). The non-linear, U-shaped, dose-response relationship with pain sensitivity has been previously documented for yohimbine (alpha-adrenoceptor agonist) and dopamine (Paalzow and Paalzow 1983; Pelissier et al. 2006). The proposed model may help to explain why the effect of *COMT* central block haplotypes is prominent with some promoter P2 haplotypes (e.g. low transcription in presence of haplotype A3 at promoter P2) and non-significant in others. Additionally, in our study haplotype A3 (previously shown to be associated with reduced transcription of *COMT*) (Bray et al. 2003) had significant main effect on pain outcomes, resulting in substantially higher pain interference in A3/A3 homozygous individuals.

In this study we also observed marked sexual dimorphism in the influence of *COMT* genotypes on pain outcomes after motor vehicle collision. The strong effect of the B2 haplotype (covering intron 5, exon 6, and 3'-untranslated region) on pain outcomes was present only in males. This finding is in accordance with previous studies showing differences in males and females in *COMT* function: *COMT* activity in the prefrontal cortex, the brain region responsible for cognitive and emotional processing of pain, has been shown to be higher in males than in females (Chen et al. 2004). These observations are also supported by animal studies, which found that knocking out the *COMT* gene results in increased dopamine in the frontal cortex in males, but not in females (Gogos et al. 1998). Additionally, genetic variations in *COMT* have also been shown to have sexually-dimorphic effects in several neuropsychiatric conditions. *COMT* Val158Met (rs4680) genotype has been found to influence the risk of obsessive-compulsive disorder (Pooley et al. 2007; Katerberg et al. 2010) and Intelligence Quotient (Barnett et al. 2009) in males but not in females, and *COMT* genotype (rs737865 and rs165599) has been found to influence schizophrenia in females, but not in males (Shifman et al. 2002; Talkowski et al. 2008). While the sexually dimorphic effects of *COMT* have been attributed to estrogen (a potent

inhibitor of *COMT* transcription) (Jiang et al. 2003), in our subgroup analyses of the participants age 55–65 effect size differences between men and women were unchanged. This suggests that the sexually dimorphic effects of haplotype B2 may not be due to estrogen alone.

One of the limitations of our study is that we did not utilize ancestry informative markers to control for population stratification in our study. However, self-reported race or ethnicity has been shown to be an accurate means of determining European American ancestry in genetic association studies (Tang et al. 2005). Additionally, all assessed polymorphisms were in Hardy-Weinberg equilibrium, suggesting little, if any, stratification in the studied samples. Adjustment for study site in the regression models was also used to minimize the effect of population variations across geographic regions of the Eastern United States. In addition, the results of our study are generalizable only to European Americans, and the influence of *COMT* genotype on pain outcomes after other traumatic events is unknown. Also, as reflected by our use of the term “persistent pain” throughout the manuscript, while six weeks of pain is an important health outcome in itself, pain of this duration does not yet meet the definition of chronic (i.e. pain lasting 2–3 months (Wolfe et al. 1990; Macrae and Davies 1999)). Future studies should evaluate the influence of *COMT* genotype on chronic pain, both after MVC and after other types of trauma exposure.

Finally, although we present no replication of our finding of the interaction between *COMT* haplotypes, our results are in strong agreement with previous literature on the role of catechol-O-methyltransferase and its polymorphisms in pain perception and chronic pain development (Diatchenko et al. 2005; Nackley et al. 2006a; Meyer-Lindenberg et al. 2006; Shifman et al. 2002; Gaysina et al. 2013; Shibata et al. 2009).

In conclusion, this is the first report of the association between *COMT* genetic variants and persistent pain and pain interference after MVC. Our study confirms that the effect of *COMT* polymorphisms on persistent pain is best evaluated using a haplotype-based approach that takes into account interactions between haplotypes in the distal promoter, coding, and 3'-untranslated region, and sex differences. Further studies are needed to investigate the biological substrate for these interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, part of the National Institutes of Health, under Award Number R01AR056328 to Dr. Samuel A. McLean. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Figure 1.
(A) *COMT* haploblocks and linkage disequilibrium; color and numbers represent D' values (dark red=high inter-SNP D' ; blue=statistically ambiguous high D' ; white= statistically ambiguous low inter-SNP D'). (B) *COMT* haplotypes and haplotype frequencies; thin lines denote haplotype combinations with frequency >1%, thick lines with frequency > 10%.

Figure 2. Haplotype A3 x LPS interactions on overall pain (A) and pain interference (B) at week 6 after motor vehicle collision; plotted values are means $\pm$ standard errors of mean; p-values are from the additive genetic model.

Figure 3. Haplotype B2 x sex interactions on overall pain (A) and pain interference (B) at week 6 after motor vehicle collision. Plotted values are means $\pm$ standard errors of mean.

Figure 4.

Hypothesized mechanistic explanation of the interaction between P2 promoter and *COMT* coding region haplotypes. (A) In presence of the *COMT* promoter P2 haplotype associated with low levels of *COMT* transcription, the effect of the haplotypes covering the coding region of *COMT* is prominent because of the steep dose-response curve; (B) In absence of the “low transcription” P2 haplotype the total enzymatic activity is shifted to the right so that the effect of the haplotypes in the coding gene region is reduced because of the flattened dose-response curve.

Table 1

Characteristics of the study participants

Characteristic	Baseline (n=948)	Lost to week 6 follow-up (n=89)	Week 6 follow-up (n=859)
Mean age, yrs (SD) ^a	36 (13)	31 (11)	36 (13)
Range	18–65	18–61	18–65
Females, n (%) ^a	575 (61)	37 (42)	538 (63)
Education, n (%) ^a			
8–11 yrs	42 (4)	6 (7)	36 (4)
HS	184 (19)	27 (31)	157 (18)
Post-HS	57 (6)	5 (6)	52 (6)
Some college	311 (33)	34 (39)	278 (32)
College	237 (25)	13 (15)	225 (26)
Post-college	113 (12)	3 (3)	110 (13)
Overall pain score (SD)	5.5 (2.4)	5.6 (2.5) ^b	3.8 (2.8)
Pain severity, n (%)			
None or mild (0–3 NRS)	188 (20)	16 (18) ^b	417 (49)
Moderate (4–6 NRS)	407 (43)	37 (42) ^b	273 (32)
Severe (7–10 NRS)	344 (37)	36 (40) ^b	168 (20)
Pain interference (SD)			
Total	-	-	16.8 (18.1)
General activity	-	-	2.8 (3.1)
Walking ability	-	-	1.5 (2.6)
Mood	-	-	2.9 (3.1)
Relations with other people	-	-	1.5 (2.7)
Sleep	-	-	2.9 (3.3)
Enjoyment of life	-	-	2.3 (3.1)

^a these characteristics were significantly different between those for whom 6 week data were obtained and those who were lost to follow-up ($p < 0.05$ for t-test or Fisher exact test, as appropriate);

^b baseline measurements; SD, standard deviation; HS, high school; NRS, numeric rating scale

Association of *COMT* haplotypes with pain and pain interference at week 6 after motor vehicle collision

Table 2

Haplotype	Number of haplotype alleles			F-value	P-value ^a
	0	1	2		
Overall pain (SE)					
A1 (CTA)	3.7 (0.2)	4.0 (0.1)	3.8 (0.2)	0.71	0.489
A2 (CTG)	3.9 (0.1)	3.8 (0.2)	4.0 (0.4)	0.29	0.749
A3 (TCG)	3.8 (0.1)	4.0 (0.2)	3.1 (0.4)	2.96	0.051
LPS (CGGG)	3.8 (0.2)	4.1 (0.1)	3.3 (0.2)	4.26	0.014
HPS (CCGG)	3.8 (0.1)	4.1 (0.2)	4.1 (0.9)	0.74	0.473
APS1 (TCAA)	3.8 (0.1)	4.0 (0.2)	3.3 (0.3)	2.58	0.077
APS2 (TCAG)	3.8 (0.1)	3.9 (0.2)	4.0 (0.4)	0.09	0.912
B1 (GA)	3.7 (0.4)	4.0 (0.2)	3.8 (0.1)	0.70	0.491
B2 (AG)	3.8 (0.1)	4.2 (0.3)	5.5 (1.2)	1.73	0.175
B3 (GG)	3.9 (0.1)	3.9 (0.2)	3.4 (0.4)	0.56	0.566
Pain interference (SE)					
A1 (CTA)	17 (1)	18 (1)	16 (1)	0.42	0.651
A2 (CTG)	17 (1)	17 (1)	18 (2)	0.04	0.965
A3 (TCG)	16 (1)	19 (1)	11 (2)	5.54	0.004 ^b
LPS (CGGG)	17 (1)	19 (1)	14 (2)	3.69	0.024
HPS (CCGG)	17 (1)	17 (2)	26 (6)	1.04	0.349
APS1 (TCAA)	17 (1)	18 (1)	15 (2)	1.46	0.230
APS2 (TCAG)	17 (1)	17 (1)	18 (3)	0.01	0.994
B1 (GA)	15 (2)	18 (1)	17 (1)	0.42	0.657
B2 (AG)	17 (1)	17 (2)	40 (8)	3.85	0.021
B3 (GG)	18 (1)	17 (1)	14 (3)	0.42	0.416

SE, standard error of mean;
^a genotypic model;
^b p-value is significant after adjustment for multiple comparisons

Table 3
Multivariate regression analysis of pain outcomes at week 6 after motor vehicle collision

Model term ^a	DF	Overall pain						Pain interference					
		Full model			Reduced model			Full model			Reduced model		
		Type III SS	F Value	P Value	Type III SS	F Value	P Value	Type III SS	F Value	P Value	Type III SS	F Value	P Value
A3	1	54.4	7.27	0.0072	49.5	6.63	0.0102	3532.3	11.25	0.0008	3942.9	12.55	0.0004
LPS	1	3.7	0.49	0.4823	8.9	1.19	0.2759	58.6	0.19	0.6659	548.8	1.75	0.1866
A3*LPS	1	83.8	11.21	0.0008	73.3	9.8	0.0018	4739.9	15.09	0.0001	4957.7	15.78	<.0001
B2	1	68.1	9.11	0.0026	66.3	8.87	0.0030	4960.1	15.79	<.0001	5657.5	18.01	<.0001
A3*B2	1	8.7	1.17	0.2801	--	--	--	8.9	0.03	0.8664	--	--	--
LPS*B2	1	0.4	0.05	0.8177	--	--	--	33.4	0.11	0.7445	--	--	--
A3*Sex	1	17.2	2.3	0.1296	--	--	--	1257.0	4	0.0458	--	--	--
LPS*Sex	1	9.7	1.29	0.2562	--	--	--	268.8	0.86	0.3551	--	--	--
B2*Sex	1	52.1	6.97	0.0085	54.5	7.29	0.0071	4861.0	15.48	<.0001	5263.1	16.75	<.0001
Sex	1	60.3	8.06	0.0046	143.6	19.21	<.0001	1187.4	3.78	0.0522	1465.3	4.66	0.0311
Site	7	104.3	1.99	0.0533	106.6	2.04	0.0480	5536.8	2.52	0.0144	5611.1	2.55	0.0133

^a A3 is a haplotype TCG from haploblock 1; LPS is a haplotype CGGG from haploblock 2; B2 is a haplotype AG from haploblock 3; Sex was coded as male=1 and female=2