Hippocampal volume, \textit{FKBP5} genetic risk alleles, and childhood trauma interact to increase vulnerability to chronic multisite musculoskeletal pain

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Chronic multisite musculoskeletal pain (CMP) is common and highly morbid. However, vulnerability factors for CMP are poorly understood. Previous studies have independently shown that both small hippocampal brain volume and genetic risk alleles in a key stress system gene, \textit{FKBP5}, increase vulnerability for chronic pain. However, little is known regarding the relationship between these factors and CMP. Here we tested the hypothesis that both small hippocampal brain volume and \textit{FKBP5} genetic risk, assessed using the tagging risk variant, \textit{FKBP5} rs3800373, increase vulnerability for CMP.

We used participant data from 36,822 individuals with available genetic, neuroimaging, and chronic pain data in the UK Biobank study. Although no main effects were observed, the interaction between \textit{FKBP5} genetic risk and right hippocampal volume was associated with CMP severity ($\beta = -0.020$, $p_{\text{raw}} = 0.002$, $p_{\text{adj}} = 0.01$). In secondary analyses, severity of childhood trauma further moderated the relationship between \textit{FKBP5} genetic risk, right hippocampal brain volume, and CMP ($\beta = -0.081$, $p = 0.016$). This study provides novel evidence that both \textit{FKBP5} genetic risk and childhood trauma moderate the relationship between right hippocampal brain volume and CMP. The data increases our understanding of vulnerability factors for CMP and builds a foundation for further work assessing causal relationships that might drive CMP development.

Up to one-third of individuals globally suffer from chronic pain in their lifetime\textsuperscript{1-3}. Of those individuals experiencing chronic pain, a substantial subset have musculoskeletal pain in multiple sites across the body\textsuperscript{4-6}. Such chronic multisite musculoskeletal pain (CMP) is highly severe\textsuperscript{7,8}, is one of the most common reasons for years lived with disability\textsuperscript{9-11}, contributes to high health care costs\textsuperscript{12,13}, and can lead to opioid addiction/abuse\textsuperscript{14,15}. Despite the substantial burden that CMP has on the individual and society, vulnerability factors for CMP and the interplay between these factors are poorly understood. This lack of understanding precludes the development of effective diagnostic tools and impedes the identification of promising targets for therapeutic intervention.

Increasing evidence indicates that two highly influential vulnerability factors for chronic musculoskeletal pain are smaller hippocampal volumes\textsuperscript{16} and the presence of one or more genetic risk alleles in a key stress system gene, \textit{FKBP5}\textsuperscript{17,18}. While the data supporting these vulnerability factors originate from independent studies, multiple lines of evidence suggest that these two risk factors are related and might interact to influence CMP development. First, both the hippocampus and \textit{FKBP5} are involved in the regulation of the physiological response to stress\textsuperscript{19-23}. Second, these two vulnerability factors have been shown to influence chronic pain states in both human and animal studies\textsuperscript{24-28}. Third, \textit{FKBP5} gene expression increases in the hippocampus in response to stress.
mean volumes \( t = 44.095, p = 2.2 \times 10^{-16} \), we assessed for associations between these brain regions and \( \text{FKBP5} \) and risk alleles in \( \text{CMP} \) is early life adversity via childhood trauma\(^{35,36} \). Previous data indicates that individuals with a history of smaller hippocampal volumes\(^ {40,41} \) and genetic/molecular risk in the vulnerability factors for \( \text{CMP} \), individuals with a history of trauma and adverse sequelae have been shown to have pants \( n = 36,822 \); 54\% women) are shown in Table 1. The majority of individuals self-identified as White \( 97\% \), genetic, brain imaging, and chronic pain data and were included in the current study. Characteristics of partici-
pants \( n = 36,822 \), had at least some college education, and were overweight \( \text{BMI} > 25 \). The UK Biobank cohort consisted of 502,487 participants, of which 36,822 participants had genetic, brain imaging, and chronic pain data and were included in the current study. Characteristics of participants \( n = 36,822 \); 54\% women) are shown in Table 1. The majority of individuals self-identified as White \( 97\% \), were more than sixty years of age, had at least some college education, and were overweight \( \text{BMI} > 25 \). The three primary hypotheses, as submitted in our UK Biobank proposal\(^ {45} \), were that (a) hippocampal brain volume, (b) \( \text{FKBP5rs3800373} \) genetic risk, and (c) the interaction between these two biological factors are associated with \( \text{CMP} \). To test these hypotheses, we first examined the relationship between \( \text{FKBP5rs3800373} \) genetic risk and \( \text{CMP} \) severity, defined by the number body sites with musculoskeletal pain for \( \geq 3 \) months. In the UK Biobank cohort used here, 32.7\% \( n = 12,038 \) of individuals reported one or more sites of \( \text{CMP} \), with the average number of sites of \( \text{CMP} \) equal to 0.48 \( \text{SD} = 0.81 \) (Supplementary Table 1). Using linear regression modeling, we found that \( \text{FKBP5rs3800373} \) genetic risk was not statistically significantly associated with \( \text{CMP} \) \( \beta = -0.003, p = 0.701 \); Supplementary Table 2). We next examined the relationship between hippocampal volume and \( \text{CMP} \) severity. Because previous literature has indicated that the left and right hippocampi play different roles in the development of pain\(^ {34,26,46} \), and because these two brain regions had different mean volumes \( t = 44.095, p = 2.2 \times 10^{-16} \), we assessed for associations between these brain regions and \( \text{CMP} \) severity independently. As shown in Supplementary Table 3, we found that neither left \( \beta = -0.005, p = 0.238 \) nor right \( \beta = -0.007, p = 0.130 \) hippocampal volumes were directly associated with \( \text{CMP} \).
rs1360780 (linkage disequilibrium with supplementary Table 9). Of note, because a second functional SNP, FKBP5, significant inverse relationship between right hippocampal volume and CMP (β = −0.016, p = 0.022). Finally in FKBP5 rs3800373 risk allele, there was a less strong but statistically significant relationship between right hippocampal brain volume and CMP (β = 0.004, p = 0.639). In those individuals with one rs3800373 risk allele, we detected no statistically significant relationship between FKBP5 volume is associated with CMP severity. Here, as shown in Table 2, we found that the interaction between FKBP5rs3800373 genetic risk and right hippocampal brain volume on CMP severity such that individuals with two FKBP5rs3800373 risk alleles showed the strongest inverse relationship between right hippocampal volume and CMP (i.e. smaller right hippocampal volume was associated with increased CMP severity; β = −0.031, p = 0.045; Supplementary Table 4). In those individuals with one FKBP5rs3800373 risk allele, there was a less strong but statistically significant inverse relationship between right hippocampal volume and CMP (β = −0.016, p = 0.022). Finally in individuals with zero FKBP5rs3800373 risk alleles, we detected no statistically significant relationship between right hippocampal volume and CMP (β = 0.004, p = 0.639).

A statistically significant interaction between FKBP5rs3800373 genetic risk and right hippocampal brain volume was associated with CMP severity in UKBB study participants. We next assessed our third hypothesis, that the interaction between FKBP5rs3800373 and hippocampal brain volume is associated with CMP severity. Here, as shown in Table 2, we found that the interaction between FKBP5rs3800373 genetic risk and right hippocampal brain volume (β = −0.020,  p_{raw} = 0.002,  p_{adj} = 0.01), but not left hippocampal brain volume (β = −0.015,  p_{raw} = 0.023,  p_{adj} = 0.12) was statistically significantly associated with CMP. The interaction explained 0.01% of the variance in CMP. Further, as shown in Fig. 1, we identified a dose-dependent inverse relationship between the number of FKBP5rs3800373 risk alleles and the relationship between right hippocampal brain volume on CMP severity such that individuals with two FKBP5rs3800373 risk alleles showed the strongest inverse relationship between right hippocampal volume and CMP (i.e. smaller right hippocampal volume was associated with increased CMP severity; β = −0.031, p = 0.045; Supplementary Table 4). In those individuals with one FKBP5rs3800373 risk allele, there was a less strong but statistically significant inverse relationship between right hippocampal volume and CMP (β = −0.016, p = 0.022). Finally in individuals with zero FKBP5rs3800373 risk alleles, we detected no statistically significant relationship between right hippocampal volume and CMP (β = 0.004, p = 0.639).

Secondary analyses identify childhood trauma as a moderator of the interaction between FKBP5rs3800373 genetic risk and right hippocampal brain volume on CMP. Numerous previous studies have identified significant gene-by-environment interactions between FKBP5 risk alleles and significant stressors47,48. Therefore, in extension to the above findings, via secondary analyses, we assessed whether distress in the form of early childhood trauma moderates the relationship between FKBP5rs3800373/right hippocampal volume and CMP (Supplementary Fig. 2). We performed this analysis in a sub-cohort of UKBB participants with available childhood trauma data (n = 25,280). Because this subset only included a portion of the full cohort used for genetic and brain volume analyses, we first assessed whether the above identified relationships remained significant in the sub-cohort. Consistent with the above findings, in this sub-cohort we found no direct association between FKBP5rs3800373 genetic risk and CMP (β = −0.001, p = 0.853; Supplementary Table 5), between left hippocampal brain volume and CMP (β = −0.005, p = 0.401; Supplementary Table 6), or between right hippocampal brain volume and CMP (β = −0.006, p = 0.285; Supplementary Table 6). Additionally, consistent with above results, we identified a statistically significant interaction between FKBP5rs3800373 genetic risk and right hippocampal volume on CMP (β = −0.017, p = 0.032; Supplementary Table 7). We then examined the interaction between childhood trauma severity and right hippocampal volume on CMP in participants with 0, 1, or 2 FKBP5rs3800373 risk alleles. The interaction was statistically significant in participants with 1 (β = −0.084, p = 0.006; Table 3) or 2 (β = −0.168, p = 0.042; Table 3) FKBP5rs3800373 risk alleles such that in individuals with higher levels of childhood trauma and FKBP5rs3800373 genetic risk, decreased hippocampal volume was associated with increased CMP (Fig. 2). For individuals with 1 or 2 risk alleles, the interaction explained 0.58% and 0.30% of the variance in CMP, respectively. No interaction between the level of childhood trauma and right hippocampal volume was observed in those without an FKBP5rs3800373 risk allele (β = −0.020, p > 0.05; Table 3). Further, when we assessed an interactive relationship between these three factors, right hippocampal volume, FKBP5rs3800373 genetic risk, and childhood trauma severity on CMP, we identified a statistically significant relationship between the interaction of these three factors and CMP (β = −0.064, p = 0.047; Supplementary Table 8) that persisted when accounting for symptoms of anxiety and depression (β = −0.081, p = 0.016; Supplementary Table 9). Of note, because a second functional SNP, FKBP5rs1360780 (linkage disequilibrium with

| Left hippocampus | Right hippocampus |
|------------------|-------------------|
| β                | S.E.              | p value | β                | S.E.              | p value |
| Intercept        | −0.302            | 0.047   | < 0.001          | −0.299            | 0.047   | < 0.001 |
| FKBP5rs3800373   | −0.003            | 0.007   | 0.692            | −0.003            | 0.007   | 0.694   |
| hippocampal volume | 0.003            | 0.006   | 0.638            | 0.004            | 0.006   | 0.476   |
| Age              | 0.001             | 0.0006  | 0.057            | 0.001             | 0.0006  | 0.065   |
| Sex              | −0.116            | 0.009   | < 0.001          | −0.117            | 0.009   | < 0.001 |
| BMI              | 0.029             | 0.001   | < 0.001          | 0.029             | 0.001   | < 0.001 |
| Genotyping array | 0.011             | 0.014   | 0.461            | 0.011             | 0.014   | 0.444   |
| Imaging center (Reading) | −0.020         | 0.013   | 0.137            | −0.020            | 0.013   | 0.135   |
| Imaging center (Newcastle) | 0.026         | 0.010   | 0.012            | 0.026             | 0.010   | 0.013   |
| FKBP5rs3800373* hippocampal volume | −0.015        | 0.007   | 0.023            | −0.020            | 0.007   | 0.002   |

Table 2. Linear regression analyses assessing the interaction between FKBP5 genetic risk, using the tagging allele rs3800373, and left and right hippocampal volumes on chronic multisite musculoskeletal pain in the UK Biobank (n = 36,822). Chronic multisite musculoskeletal pain was defined as the number of musculoskeletal sites with pain that persisted for more than 3 months, ranging from 0 to 4. An additive genetic model was used to assess the effect of rs3800373. Genetic principal components 1–10 and ethnic background were included as covariates but omitted from the table for brevity.
**Figure 1.** The number of FKBPsrs3800373 risk alleles moderates the relationship between the right hippocampus and CMP severity. Shown is the relationship between right hippocampal brain volume and chronic multisite musculoskeletal pain (CMP) severity in individuals from the UKBB study cohort (n = 36,822). Right hippocampal brain volume was adjusted for head size and standardized (mean = 0, SD = 1), see “Methods”. Individuals with two copies of the FKBPsrs3800373 risk allele (n = 3005) are represented by the blue line, individuals with one copy of the FKBPsrs3800373 risk allele (n = 15,327) are represented by the red line, and individuals with no risk alleles are represented by the green line (n = 18,490). As shown in Supplementary Table 4, relationships represented by the blue and red lines were statistically significant (p < 0.05).

**Table 3.** Linear regression analyses assessing the interaction between childhood trauma and right hippocampal volume on chronic multisite musculoskeletal pain in individuals with 0, 1, or 2 FKBPsrs3800373 risk alleles (n = 25,280). Chronic multisite musculoskeletal pain was defined as the number of musculoskeletal sites with pain that persisted for 3 months or more, ranging from 0 to 4 sites. Genetic principal components 1–10 and ethnic background were included as covariates but omitted from the table for brevity.

|                      | 0 risk alleles (n = 12,777) | 1 risk allele (n = 10,464) | 2 risk alleles (n = 2,039) |
|----------------------|-----------------------------|----------------------------|-----------------------------|
|                      | β   | S.E. | p       | β   | S.E. | p       | β   | S.E. | p       |
| Intercept            | −0.311 | 0.078 | < 0.001 | −0.260 | 0.084 | 0.002 | −0.301 | 0.188 | 0.110 |
| Right hippocampal volume | 0.004 | 0.008 | 0.577 | −0.012 | 0.008 | 0.158 | −0.014 | 0.019 | 0.452 |
| Childhood trauma severity | 0.192 | 0.028 | < 0.001 | 0.189 | 0.032 | < 0.001 | 0.178 | 0.067 | 0.008 |
| Age                  | 0.0008 | 0.001 | 0.399 | 0.0007 | 0.001 | 0.520 | 0.002 | 0.002 | 0.331 |
| Sex                  | −0.103 | 0.014 | < 0.001 | −0.105 | 0.016 | < 0.001 | −0.078 | 0.035 | 0.027 |
| BMI                  | 0.029 | 0.002 | 0.002 | 0.027 | 0.002 | < 0.001 | 0.022 | 0.004 | < 0.001 |
| Genotyping array     | 0.020 | 0.024 | 0.408 | 0.001 | 0.026 | 0.972 | 0.0347 | 0.059 | 0.427 |
| Imaging center (Reading) | −0.029 | 0.022 | 0.177 | 0.017 | 0.024 | 0.489 | 0.034 | 0.054 | 0.528 |
| Imaging center (Newcastle) | 0.004 | 0.018 | 0.803 | 0.035 | 0.019 | 0.074 | 0.152 | 0.043 | < 0.001 |
| Right hippocampal volume*childhood trauma severity | −0.020 | 0.028 | 0.470 | −0.084 | 0.031 | 0.006 | −0.168 | 0.083 | 0.042 |

FKBPsrs3800373, $D’ = 0.89, r^2 = 0.78$) has been shown to be associated with childhood trauma and is functional, we also tested the triple interaction using FKBPsrs1360780 ($\beta = -0.063, p = 0.048$; Supplementary Table 10).

**Discussion**

Here we show for the first time and using the largest sample size to date that includes genetic, neuroimaging, and chronic pain data, that vulnerability to CMP is highest in individuals with a combination of FKBPsrs3800373 genetic risk alleles and smaller right hippocampal brain volumes. Further, we show that increased exposure to
Proportional volume of right hippocampus vs. childhood trauma and FKBP5 rs3800373 risk alleles moderate the relationship between the right hippocampus and CMP severity. Shown is the relationship between right hippocampal brain volume and chronic multisite musculoskeletal pain (CMP) severity in individuals from the UK Biobank study with (a) 0, (b) 1, and (c) 2 FKBP5 rs3800373 risk alleles and varying degrees of childhood trauma (n = 25,280). Right hippocampal brain volume was adjusted for head size and standardized (mean = 0, SD = 1), see “Methods”. Individuals who did not often experience childhood trauma are represented by the green line (“no trauma”), individuals who often experienced one type of childhood trauma are represented by the red line (“single trauma type”), and individuals who often experienced multiple (>2) types of childhood trauma are represented by the blue line (“multiple trauma types”). As shown in Supplementary Table 8, the interaction between FKBP5 rs3800373, right hippocampal volume, and childhood trauma was statistically significant (p < 0.05).

While the main effect relationships that were hypothesized between FKBP5 genetic risk and CMP and between hippocampal volume and CMP did not hold up in the current set of analyses (as they had in previous studies of chronic pain16,49–51), the interaction between these two factors did show association with CMP. Since both FKBP5 and the hippocampus are central to stress system functioning19–23,52,53, and the combination of these stress system mediators substantially increases vulnerability to CMP (especially in individuals with a history of substantial childhood stressors), it suggests that the physiological stress-response system is critical to the development of CMP. Such findings are important because they provide further support to existing literature indicating that therapeutic strategies aimed at reducing CMP might be more effective if targeted to stress system mediators vs tissue injury generators49,55.

The HPA axis stress system and its role in the development of neuropsychiatric disorders has been studied extensively previously48. Data from this field, in addition to nascent literature from the pain field, can be used to help generate further hypotheses to later test how the identified stress-associated vulnerability factors might be contributing to the pathogenesis of CMP. For instance, because the protein encoded by FKBP5, FKBP51, regulates the affinity of the glucocorticoid receptor (GR) to the glucocorticoid cortisol58, and the FKBP5 risk allele causes an increase in FKBP51 synthesis (thus decreasing glucocorticoid sensitivity), one could hypothesize that in tissues with a high density of glucocorticoid receptors (e.g., hippocampus56), that increased glucocorticoid resistance (e.g., in individuals with FKBP5 genetic risk and in individuals that have been exposed to substantial stressors) could cause maladaptive changes to tissue morphology (such as tissue atrophy). Such glucocorticoid-induced tissue atrophy has been observed previously via studies assessing cortisol inhibition of cellular formation and proliferation in the hippocampus56, likely via apoptosis in the subgranular zone of the dentate gyrus57. One might then hypothesize that lower glucocorticoid sensitivity and atrophied hippocampal tissue (due to glucocorticoids) lead to smaller anatomical hippocampal sizes that predispose an individual to CMP via the reduced negative feedback inhibition control of the hippocampus on the HPA axis58–61. Chronic pain poses an important biological × environmental interactions, compound risk for CMP. In addition, this work provides an important foundational framework for subsequent mechanistic work that aims to identify causal relationships between the identified factors.

Childhood trauma further extends this biological vulnerability to CMP. Importantly, these associations were not accounted for by differences in age, sex, and BMI, nor were they accounted for by symptoms of depression or anxiety. Together, these findings add to the growing body of literature indicating that multiple factors, including important biological × environmental interactions, compound risk for CMP. While substantial data, including the data presented here, supports these mechanistic hypotheses, the direct causal relationships between the identified vulnerability factors and CMP has yet to be studied.
In addition to hippocampal volume and FKBP5 genetic risk being important to the development of CMP, we also showed in this study that childhood stress via emotional, physical, and/or sexual abuse increases an individual’s vulnerability to CMP. In extension to the above mechanistic hypotheses, one might further expand upon the hypotheses to include the role that childhood trauma plays in the pathogenic mechanisms driving CMP. For instance, evidence from multiple species indicates that traumatic events in childhood alter normal development of brain structure, function and behaviour64, most notably in the hippocampus65–70. Additionally, the severity of childhood trauma and the developmental timing of this trauma influence stress responses in adulthood such that children who suffered from physical or sexual abuse in the first five years of life were more likely to have HPA axis dysregulation71. These HPA axis changes due to childhood abuse are thought to be mediated by epigenetic changes such as DNA de-methylation of FKBP572, which occurs more commonly in individuals with at least one copy of the genetic risk allele in this gene42,47. Finally, as might be relevant to individuals in the UK Biobank cohort (i.e. with an average age of ~64 in the current sub-cohort), previous studies indicate that the effect that childhood trauma has on FKBP51, the hippocampus, and HPA axis persists throughout the lifespan72–75.

Several limitations should be considered when interpreting these study results. First, the cross-sectional study design implemented here limited our ability to assess for direct causation (e.g. between an inciting traumatic event and subsequent transition from acute to chronic pain). Second, the UK Biobank cohort is comprised of mainly European/White individuals, thus the generalizability of our results to other racial/ethnic groups is not known. Third, we did not control for potential confounding by medication use (e.g. opioids) or by comorbid disorders other than frequently co-occurring neuropsychiatric conditions. Fourth, the extent of both CMP and childhood trauma in this cohort was relatively low, limiting the sample size in some stratified analyses. Fifth, since our dataset did not include severity or duration of pain, our CMP variable has a more limited characterization of pain than other studies; however, we created our CMP variable using methodology that is consistent with other research that studies pain in the UK Biobank database76–77. Finally, the presented results remain to be validated in an independent dataset.

In conclusion, we presented novel evidence that both FKBP5rs3800373 genetic risk and childhood trauma moderate the relationship between hippocampal volume and CMP. While previous evidence highlighted these factors as independent predictors of chronic musculoskeletal pain, we found here that these factors interact such that individuals with a combination of these related risk factors have the highest risk for CMP. Future studies should investigate whether these factors not only indicate vulnerability for CMP but whether they also contribute to the underlying pathogenesis of disease. Altogether, these findings increase our understanding of risk factors for CMP and identify potential targets for therapeutic intervention within the stress axis pathway.

Methods

Study design and population. The UK Biobank is a population-based cohort of over 500,000 participants that was designed to improve the prevention, diagnosis, and treatment of chronic conditions. Between 2006 and 2010, all UK residents registered with the National Health Service, aged 40–69 years, and living within 25 miles of one of the 22 assessment centers across the country, were invited to participate. At baseline assessment, participants completed a detailed touch-screen questionnaire which collected demographic, lifestyle, and medical information. Biological samples (such as blood and saliva) and physical measurements (such as height and weight) were also collected. Between 2014 and 2020, enrolled participants were invited to participate in a follow-up assessment in which they repeated the initial questionnaire and underwent brain imaging protocols. Participants also regularly responded to web-based questionnaires including a mental health questionnaire between 2016 and 2017. The current study includes 36,822 participants with genetic, brain imaging, and chronic pain data. A sub-cohort of 25,280 participants with childhood trauma data was used for secondary analyses.

Assessments. Chronic multisite musculoskeletal pain (CMP). At the follow-up visit between 2014 and 2020 (at the same time that brain imaging data was acquired), participants were asked “In the last month have you experienced any of the following that interfered with your usual activities?” The response options were seven specific sites of pain (headache, facial pain, neck or shoulder pain, back pain, stomach or abdominal pain, hip pain, and knee pain) as well as “pain all over the body”, “none of the above”, or “prefer not to say.” Participants were permitted to select multiple painful sites unless they reported having “pain all over the body,” “none of the above”, or “prefer not to say.” Participants who indicated that they had pain at specific sites were then asked whether the pain at each site had been present for more than 3 months6,9. Consistent with previous studies6,77, only the neck or shoulder, back, hip, and knee sites were used to investigate musculoskeletal pain. Participants who reported having pain all over the body were excluded since chronic widespread pain is distinct from chronic musculoskeletal pain9,80. Chronic multisite musculoskeletal pain (CMP) was defined as the number of musculoskeletal sites with pain that persisted for more than 3 months, ranging from 0 to 4.

Hippocampal brain volume. Brain images were acquired using Siemens Skyra 3 T scanners in UK Biobank’s imaging centers in Cheadle (n = 23,495), Reading (n = 4491), and Newcastle (n = 8836) using identical acquisition protocols across sites81. T1-weighted brain images were processed using FreeSurfer v6.0 to automatically estimate right and left hippocampal volumes and total intracranial volumes82–84. Hippocampal volumes were divided by total intracranial volume in order to adjust for head size85,86. Right and left hippocampal volumes were normally distributed both before and after adjustment (Supplementary Fig. 1). We used a t-test to compare the
difference in means (mean volume of right hippocampus = 3795 mm³, mean volume of left hippocampus = 3677 mm³; adjusted mean volume of right hippocampus = 0.00246 and the adjusted mean volume of left hippocampus = 0.00238). Standardized adjusted hippocampal volumes (mean = 0, SD = 1) were used in statistical models.

**FKBP5 genetic risk.** Genotyping was performed by the UK Biobank on the full cohort. In the current study, we utilized genotyping data from only those participants with brain imaging data. Genotyping was performed using the Affymetrix UK BiLEVE Axiom array (n = 3469) and the Affymetrix UK Biobank Axiom array (n = 33,353). Quality control procedures, as described previously, were performed centrally by UK Biobank personnel prior to distribution to individual investigators. In the current set of analyses we focused on a single genetic variant in the FKBP5 gene, rs3800373, based on its ability to tag the main risk haplotype and because it has been shown previously to be associated with persistent musculoskeletal pain via its allele-specific influence on microRNA regulation of FKBP5 expression. This allele was in Hardy–Weinberg equilibrium (p > 0.05) and had an excellent call rate (> 99%). Consistent with the reported minor allele frequency (MAF) of rs38003873 for the ‘British in England and Scotland’ (GBR) sub-population of European (EUR) ancestry (MAF = 32%, 1000 Genomes), the MAF for participants included in the current study was 29%. An additive genetic model (homozygous for the major allele (AA) = 0, heterozygous (CA) = 1, and homozygous for the minor allele (CC) = 2) was used to assess the relationship between FKBP5 rs3800373, hippocampal brain volume, childhood trauma and CMP. Of note, rs1360780 is in high linkage disequilibrium with rs3800373 (D² = 0.89, r² = 0.78), is associated with childhood trauma and has been shown to influence FKBP5 transcription via DNA methylation. Due to high LD between this allele and FKBP5 rs3800373, we used this allele to confirm key findings demonstrated with FKBP5 rs3800373.

**Childhood trauma.** A sample of the original cohort (n = 25,280) completed a subsequent online mental health questionnaire between 2016 and 2017 that contained questions pertaining to childhood trauma. Three questions from the Childhood Trauma Questionnaire were used to identify participants who experienced physical abuse ("When I was growing up...People in my family hit me so hard that it left me with bruises or marks"), emotional abuse ("When I was growing up...I felt that someone in my family hated me"), and sexual abuse ("When I was growing up...someone molested me (sexually)"). Possible responses were never true, rarely true, sometimes true, often true, very often true, or prefer not to say. For each question, similarly to previous definitions of frequency, participants who responded "often true" or "very often true" were said to frequently experience the dimension of childhood trauma that participants experienced.

**Depression and anxiety symptoms.** To control for neuropsychiatric conditions related to CMP, two additional items from the online mental health questionnaire were used to assess core symptoms of depression and anxiety. The question "Have you ever had a time in your life when you felt sad, blue, or depressed for two weeks or more in a row?" was used to assess lifetime depressive symptoms and the question "Have you ever had a period lasting one month or longer when most of the time you felt worried, tense, or anxious?" was used to assess anxiety symptoms. If participants had an affirmative response to the item, we considered the participant affected by the neuropsychiatric condition.

**Statistical analyses.** Sociodemographic characteristics were summarized using standard descriptive statistics. The relationship between FKBP5 rs3800373, hippocampal brain volume, and chronic multisite musculoskeletal pain in the UK Biobank cohort was assessed using general linear models. Interactions between FKBP5 rs3800373 and hippocampal volume were assessed by including corresponding product terms in the model. Based on previous analyses, age, sex, BMI, and self-reported ethnic background were included in models as covariates. Models assessing the effect of FKBP5 rs3800373 genetic risk also included genotyping array and top 10 genetic principal components as covariates. Models assessing the effect of hippocampal volume also included imaging site as a covariate, similarly to previous studies. The coefficient of determination (R², i.e. the proportion of the variation in the dependent variable that is predictable from the independent variable) of the interaction term was calculated as the difference between R² of the full model that contains the interaction term along with covariates and the R² of the null model that only includes covariates. We adjusted for multiple testing using Bonferroni correction and reported both raw and adjusted p-values. Statistical significance was determined based on adjusted p values. The sample (n = 36,822) was sufficient to test for gene-environment interactions. The minimum sample size to achieve 80% power at 5% significance level to detect a small effect size gene-environment interaction for a genetically dominant SNP with p = 0.3 frequency is > 20,000 participants.

Secondary analyses tested moderation via the interaction between childhood trauma and hippocampal volume on CMP in individuals with 0, 1, and 2 FKBP5 rs3800373 risk alleles. All analyses were performed using RStudio server (1.4.1106-5) and all graphs were made for publication using GraphPad Prism v 9.1.2.

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**Author contributions**
M.M. and S.D.L. developed the hypothesis. M.M. applied for UKBiobank data. J.J.L. and S.D.L. designed the analysis and J.J.L performed statistical models and data visualization. All authors wrote, reviewed, and edited the manuscript.

**Competing interests**
The authors declare no competing interests.

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