1   **The genetic architecture of pain intensity in a sample of 598,339 U.S. veterans**

2   Sylvanus Toikumo[1,2], Rachel Vickers-Smith[1,3], Zeal Jinwala[2], Heng Xu[2], Divya Saini[2], Emily Hartwell[1,2],

3   Mirko P. Venegas[4], Kyle A. Sullivan[4], Ke Xu[5,6], Daniel A. Jacobson[4], Joel Gelernter[5,6], Christopher T.

4   Rentsch[5,6,7], Million Veteran Program, Eli Stahl,[8] Martin Cheatle[2], Hang Zhou[5,6], Stephen G. Waxman[5,6],

5   Amy C. Justice[5,6,9], Rachel L. Kember[1,2], Henry R. Kranzler[1,2]

6

7   [1]Mental Illness Research, Education and Clinical Center, Crescenz VAMC, Philadelphia, PA, USA;

8   [2]Department of Psychiatry, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA,

9   USA; [3]Department of Epidemiology, University of Kentucky College of Public Health; Center on Drug and

10   Alcohol Research, University of Kentucky College of Medicine, Lexington, KY, USA; [4]Biosciences Division,

11   Oak Ridge National Laboratory, Oak Ridge, TN, USA; [5]Veterans Affairs Connecticut Healthcare System,

12   West Haven, CT, USA; [6]Yale University School of Medicine, New Haven, CT, USA; [7]London School of

13   Hygiene & Tropical Medicine, London, UK; [8]Regeneron Genetics Center, Tarrytown, NY, USA; [9]Yale

14   University School of Public Health, New Haven, CT, USA

15

16   **Corresponding author:** Henry R. Kranzler, MD, Department of Psychiatry, University of Pennsylvania

17   Perelman School of Medicine, Philadelphia, PA, USA. Email: kranzler@pennmedicine.upenn.edu

18

19

20

21

22

23

24

25

26   **Abstract**

27   Chronic pain is a common problem, with more than one-fifth of adult Americans reporting pain daily or

28   on most days. It adversely affects quality of life and imposes substantial personal and economic costs.

29   Efforts to treat chronic pain using opioids played a central role in precipitating the opioid crisis. Despite

30   an estimated heritability of 25-50%, the genetic architecture of chronic pain is not well characterized, in

31   part because studies have largely been limited to samples of European ancestry. To help address this

32   knowledge gap, we conducted a cross-ancestry meta-analysis of pain intensity in 598,339 participants in

33   the Million Veteran Program, which identified 125 independent genetic loci, 82 of which are novel. Pain

34   intensity was genetically correlated with other pain phenotypes, level of substance use and substance

35   use disorders, other psychiatric traits, education level, and cognitive traits. Integration of the GWAS

36   findings with functional genomics data shows enrichment for putatively causal genes (n = 142) and

37   proteins (n = 14) expressed in brain tissues, specifically in GABAergic neurons. Drug repurposing analysis

38   identified anticonvulsants, beta-blockers, and calcium-channel blockers, among other drug groups, as

39   having potential analgesic effects. Our results provide insights into key molecular contributors to the

40   experience of pain and highlight attractive drug targets.

41

42

43

44

45

46

47

48

49

50

51

52

53   **Introduction**

54   Pain is an unpleasant sensory and emotional experience associated with, or resembling that

55   associated with, actual or potential tissue damage[1]. Pain is often classified as either acute, which

56   typically lasts less than 4 weeks, and chronic, lasting more than three months and potentially

57   maladaptive[2]. An individual's experience of pain is influenced by biological, psychological, and social

58   factors[1,3].

59   In a national survey, 50.2 million US adults (20.5%) reported experiencing pain on most days or

60   every day[4], making pain the most common reason for seeking medical treatment[5] and resulting in total

61   healthcare costs of 560 to 635 billion dollars in 2010[6]. Chronic pain is also associated with a poor quality

62   of life[7]. In the late 1980's many medical and pain organizations adopted policies to increase patients'

63   access to pain management, including opioids. These policies included efforts to ensure the adequate

64   assessment of pain, which was designated as "the fifth vital sign"[2]. The resulting dramatic increase in

65   prescriptions for opioid analgesics contributed to the opioid epidemic and a doubling of opioid-related

66   deaths in the 1990s[8,9].

67   Success rates for treating chronic pain with currently available medications are estimated to be

68   as low as 10%[10]. Opioids are not efficacious in managing chronic non-cancer pain[11] and their long-term

69   use is associated with adverse effects such as addiction, sleep disturbance, opioid-induced hyperalgesia,

70   endocrine changes, and cardiac and cognitive effects[12,13]. Other medications used to treat chronic non-

71   cancer pain, such as non-steroidal anti-inflammatory medications and antiepileptic drugs, are effective

72   for only some types of pain and may be associated with significant adverse effects[14]. Because non-

73   pharmacologic interventions are not accessible to most patients with pain, safe and efficacious

74   medications are needed to address this highly prevalent condition. Thus, novel therapeutic targets for

75   chronic pain are needed to facilitate the discovery or repurposing of safe, effective analgesics.

76   Notably, drug development efforts informed by genetics can double the rate of success[15–17].

77   Although the heritability (h^2) of individual differences in the susceptibility to develop chronic pain is

78   estimated in twin and family studies to be 25–50%[18,19], the mechanisms that underlie it are poorly

79   understood[20]. To date, genome-wide association studies (GWAS) of chronic pain in large samples,

80   including the UK Biobank (UKBB) and 23andMe cohorts, have focused on specific bodily sites[21–24] or

81   aspects of an individual's sensitivity to experiencing and reporting pain[25–28]. Although in samples of

82   150,000 to nearly 500,000 individuals GWAS have identified genome-wide significant (GWS) loci for

83   headache[29], osteoarthritis[30,31], low back pain[23,24], knee pain[21], neuropathic pain[32], and multisite chronic

84   pain[25,26], they have yielded few overlapping loci. This may be due to the different pain phenotypes

85   employed, despite their having high genetic correlations among them[27,33].

86   There are also significant genetic correlations between pain phenotypes and psychiatric,

87   substance use, cognitive, anthropometric, and circadian traits[21,23–25,29,34]. This shared genetic

88   predisposition suggests that a common genetic susceptibility underlies a broad range of diverse chronic

89   pain conditions[34] and common co-occurring conditions. For example, Mendelian randomization (MR)

90   and latent causal variable analyses have shown positive causal effects of specific bodily site pain on

91   depression[35,36] and bi-directional casual associations between multisite chronic pain and major

92   depressive disorder (MDD)[25,35].

93   Despite a growing literature on pain GWAS, most studies have been conducted in predominantly

94   European ancestry cohorts recruited from non-clinical biobanks. However, biobanks linked to electronic

95   health records (EHRs) with large, well-characterized, multi-ancestry samples are now available for use in

96   identifying genetic risk factors and therapeutic targets for chronic pain[37]. The Million Veteran Program

97   (MVP)[38], an observational cohort study and mega-biobank implemented in the U.S. Department of

98   Veterans Affairs (VA) health care system, includes data on routine pain screening. Pain ratings in the

99   MVP use an 11-point ordinal Numeric Rating Scale (NRS), which has been a standard practice in VA

100   primary care for more than a decade[39]. The NRS has been shown to be a consistent, valid measure of

101   reported pain[40–42] and is particularly informative for a GWAS of pain, as over 50% of VA patients

102   experience chronic pain[43].

103   We conducted a cross-ancestry meta-analysis of the NRS in samples of African American (AA),

104   European American (EA) and Hispanic American (HA) ancestries from the MVP (N = 598,339). Because of

105   the frequency with which the NRS is administered to patients in the VA, for each individual we

106   calculated the median annual score and then the median across years. Thus, although the NRS is a

107   report of pain intensity experienced at a specific point in time, the median of medians provided a proxy

108   for chronic pain. We also conducted a secondary analysis in a subsample of 566,959 individuals that

109   excluded participants with a lifetime opioid use disorder (OUD) diagnosis to assess potential

110   confounding by OUD.

111

112   **Methods**

113   **Overview of analyses**

114   We conducted ancestry-specific GWASs of pain scores using an 11-point ordinal NRS in a) all

115   AAs, EAs, and HAs with pain ratings from the MVP and b) a subset of these participants that excluded

116   those with a lifetime OUD diagnosis, each followed by a cross-ancestry meta-analysis. Details on

117 phenotyping are provided below. Downstream analyses are based principally on the GWAS of pain

118 scores in the full sample, complemented by the estimated heritability and genetic correlations (r_gs) for

119 the sample exclusive of participants with OUD. An overview of the analyses is provided in

120 Supplementary Fig. 1.

121 **Million Veteran Program cohort**

122 The MVP[38] is an EHR-based cohort comprising >900,000 veterans recruited at 63 VA medical

123 centers nationwide. All participants provided written informed consent, a blood sample for DNA

124 extraction and genotyping, and approval to securely access their EHR for research purposes. The

125 protocol and consent were approved by the Central Veterans Affairs Institutional Review Board (IRB)

126 and all site-specific IRBs. All relevant guidelines for work with human participants were followed in the

127 conduct of the study.

128

129 **Phenotype description**

130 As early as 2000, the VA recommended using the NRS to routinely measure pain in clinical

131 practice as a "fifth vital sign"[44]. Since that time, veterans have been asked to rate their pain severity in

132 response to the question: "Are you in pain?" They then rated their current pain on a scale of 0-10 where

133 "0 is no pain and 10 is the worst pain imaginable". Participants had at least one inpatient or outpatient

134 pain rating in the EHR. We included 598,339 individuals with 76,798,104 NRS scores (median number of

135 scores = 109, IQR = 28 – 351) in the primary GWAS. To reduce the large number of pain observations, we

136 calculated the median pain score by year for each participant and the median of the annual median pain

137 scores. In a supplementary GWAS we excluded individuals with a documented ICD-9/10 diagnosis code

138 for OUD in the EHR, yielding a total of 566,959 study participants. Demographic characteristics for the

139 supplementary sample are presented in Supplementary Table 1.

140

141 **Genotyping and imputation**

142 DNA samples were genotyped on the Affymetrix Axiom Biobank Array (MVP Release 4). For

143 genotyped SNPs, standard quality control (QC) and subsequent imputation were implemented. Full

144 details about SNP and sample QC by the MVP Genomics Working Group are published[45]. Briefly, DNA

145 samples were removed for sex mismatch, having seven or more relatives in MVP (kinship > 0.08),

146 excessive heterozygosity, or genotype call rate < 98.5%. Variants were removed if they were

147 monomorphic, had a high degree of missingness (call rate < 0.8) or a Hardy–Weinberg equilibrium

148 (HWE) threshold of $P < 1 \times 10^{-6}$ both in the entire sample using a principal-component analysis (PCA)-

149 adjusted method and within one of the three major ancestral groups (AA, EA and HA).

150 Genotype phasing and imputation were performed using SHAPEIT4 (v.4.1.3)[46] and Minimac4

151 software[47], respectively. Biallelic SNPs were imputed using the African Genome Resources reference

152 panel by the Sanger Institute (comprising all samples from the 1000 Genomes Project phase 3, version 5

153 reference panel[48], and 1,500 unrelated pan-African samples). Non-biallelic SNPs and indels were

154 imputed in a secondary imputation step using the 1000 Genomes Project phase 3, version 5 reference

155 panel[48], with indels and complex variants from the second imputation merged into the African Genome

156 Resources imputation.

157 We removed one individual from each pair of related individuals (kinship > 0.08, $N = 31,010$)

158 at random. The HARE method[49] was used to classify subjects into major ancestral groups (AA = 112,968,

159 EA = 436,683, HA = 48,688) and QC of imputed variants was performed within each ancestral group.

160 SNPs with imputation quality (INFO) score < 0.7; minor allele frequency (MAF) in AAs < 0.005,

161 EAs < 0.001, and HAs < 0.01; a genotype call rate < 0.95; or an HWE $P < 1 \times 10^{-6}$ were excluded.

162 **Association analyses and risk locus definition**

163 Genome-wide association testing was based on a linear regression model using PLINK (v.2.0)[50]

164 and was adjusted for sex, age at enrollment, and the first 10 within-ancestry genetic principal

165 components (PCs). Due to substantial differences in sample size across ancestral groups, meta-analyses

166 were performed using a sample-size weighted method in METAL[51]. Variants with $P < 5 \times 10^{-8}$ were

167 considered genome-wide significant (GWS).

168 To identify risk loci and their lead variants, we performed LD clumping in FUMA[52] at a range of

169 3,000 kb, $r^2 > 0.1$, and the respective ancestry 1000 Genomes reference panel[48]. Following clumping,

170 genomic risk loci within 1 Mb of one another were incorporated into the same locus. We used GCTA

171 COJO[53] to define independent variants by conditioning them on the most significant variant within the

172 locus. After conditioning, significant variants ($P < 5 \times 10^{-8}$) were considered independently associated.

173 We performed a sign test to compare the direction of SNP effects across individual ancestral datasets.

174 Independent lead variants in EAs were examined in AAs and HAs and a binomial test used to evaluate

175 the null hypothesis that 50% of variants have the same effect direction across ancestries. For lead SNPs

176   in EAs that were absent in AAs and HAs, we considered proxy GWS SNPs ($p < 5×10^{-8}$) in high LD with the

177   EA lead variant (r^2 ≥0.8).

178   To prioritize credible sets of variants driving our GWAS results, we used FINEMAP[54] to fine-map

179   regions defined by LD clumps ($r^2 > 0.1$). Because fine-mapping requires data from all markers in the

180   region of interest[55], we merged LD clumps that physically overlapped (within a 1-MB window of the lead

181   variant) and excluded SNPs in the major histocompatibility complex (MHC) region due to its complexity.

182   FINEMAP credible set reports the likelihood of causality using the marginal posterior probability (PP),

183   which ranges from 0 to 1, with values closer to 1 being most likely causal.

184   **SNP-based heritability and functional enrichment**

185   We used the linkage disequilibrium score (LDSC) regression[56] method to estimate the SNP-based

186   heritability (h^2_{SNP}) of pain intensity (in both the full and the supplementary samples) in AAs and EAs

187   based on common SNPs in HapMap3[57]. Due to the small HA sample size, we could not calculate h^2_{SNP} in

188   this population. To ensure matching of population LD structure, pre-calculated LD scores for EAs were

189   derived from the 1000 Genomes European reference population (version 3)[49] using LDSC[56]. In-sample LD

190   scores for AAs were calculated from MVP AA genotype data using cov-LDSC[58].

191   We used S-LDSC to partition the SNP heritability for pain intensity among EAs and explored the

192   enrichment of the partitioned heritability by functional genomic categories[59,60] using three models: (a) a

193   baseline-LD model that contains 75 overlapping annotations, including coding and regulatory regions of

194   the genome and epigenomic features[59] (b) a specific tissue model that examines 10 overlapping cell-

195   type groups derived from 220 cell-type-specific histone marks, including methylated histone H3 Lys4

196   (H3K4me1), trimethylated histone H3 Lys4 (H3K4me3), acetylated histones H3 Lys4 (H3K4ac) and

197   H3K27ac[59] and (c) a multi-tissue model based on gene expression and chromatin datasets generated by

198   GTEx[61] and the Roadmap Epigenomics Mapping Consortium[62]. For each model, we excluded multi-allelic

199   and MHC region variants. Functional categories within each model were considered significantly

200   enriched based on a Bonferroni-corrected P value.

201   **Gene-set functional characterization**

202   We applied multi-marker analysis of genomic annotation (MAGMA) v.1.08[63] in FUMA (v1.3.6a)[52]

203   to identify genes and gene sets associated with the findings from the pain intensity GWAS and meta-

204   analysis. Using the default setting in MAGMA, we mapped GWS SNPs to 18,702 protein-coding genes

205   according to their physical position in NCBI build 37. We also used chromatin interaction (Hi-C) coupled

206   MAGMA (H-MAGMA)[64] to assign non-coding (intergenic and intronic) SNPs to genes based on their

207   chromatin interactions. H-MAGMA uses six Hi-C datasets derived from fetal brain, adult brain (N = 3),

208   induced pluripotent stem cell (iPSC)-derived neurons and iPSC-derived astrocytes[65]. We applied a

209   Bonferroni correction (MAGMA, α = 0.05/18,702; H-MAGMA, α = 0.05/293157/6) to identify genes

210   significantly associated with pain intensity, correcting for all genes tested in each analysis (see

211   Supplementary Tables 15 and 21 for full lists).

212   To determine the plausible tissue enrichment of mapped genes, we integrated our cross-

213   ancestry and EA GWAS results with gene expression data from 54 tissues (GTEx v8) in FUMA[52]. Next, we

214   used FUMA to curate gene sets and Gene Ontology terms (from the Molecular Signature Database

215   v.7.0[66]). We corrected for gene size, density of variants, and LD pattern between genes in each tissue

216   (Bonferroni-corrected α = 0.05/54).

217   Enrichment for cell-type specific (CTS) transcriptomic profiles was performed in FUMA[67] using 13

218   human single-cell RNA-sequencing (sc-RNAseq) datasets derived from brain (see Supplementary Table

219   14 for a detailed list). FUMA estimates CTS transcriptomic enrichment from the sc-RNAseq in three

220   ways: (1) per selected dataset, (2) within datasets using a conditionally independent analysis (based on

221   stepwise conditional testing of P values for each cell type that passes Bonferroni correction within the

222   same dataset), and (3) across datasets (testing for proportional significance across the results from step

223   2). Proportional significance (PS) reports the confidence level for observed cell type enrichment as low

224   significance: < 0.5, jointly significant: 0.5 – 0.8; and independently significant: > 0.8. We considered CTS

225   enrichments with conditional independent signals (P < 0.05) and PS > 0.5 to be driven by

226   joint/independent genetic signals in our pain intensity GWAS results.

227   **Transcriptomic and proteomic regulation**

228   To identify genes and proteins whose expression is associated with pain intensity, we integrated

229   EA GWAS results with human brain transcriptomic (eQTL, N = 452; and sQTL, N = 452)[68,69] and proteomic

230   (N = 722)[62] data. We also obtained pretrained models of gene expression from GTEx v.8 for five brain

231   tissues significantly enriched in MAGMA analyses – cerebellum, cerebellar hemisphere, cortex, frontal

232   cortex, and anterior cingulate cortex[61,71]. Human brain transcriptomic and proteomic data for

233   dorsolateral prefrontal cortex were derived from the study by Wingo et al[70]. Transcriptome-wide

234   association study (TWAS) and proteome-wide association study (PWAS) analyses were performed using

235   the FUSION pipeline[71] with Bonferroni correction (α = 0.05/N genes tested) to account for multiple

236   testing.

237   We used the colocalization (coloc R package[72] in FUSION[71]) as our primary method to identify

238   SNPs that mediate association with pain intensity through effects on gene and protein expression and a

239   posterior colocalization probability (PP) of 80% to denote a shared causal signal. To test the robustness

240   of the colocalized signals, we also performed summary-based Mendelian randomization (SMR)

241   analyses[73]. We applied the HEIDI test[73] to filter out SMR signals (P_{HEIDI} < 0.05) due to linkage

242   disequilibrium between pain-associated variants and eQTLs/sQTLs. Human brain cis-eQTL and cis-sQTL

243   summary data were obtained from Qi et al[74] and GTEx[61]. For genomic regions containing multiple genes

244   with significant SMR associations, we selected the top-associated cis-eQTL. We used Bonferroni

245   correction to correct for multiple testing (α = 0.05/N genes tested) .

246   To explore the enrichment of causal genes and proteins in the dorsal root ganglia (DRG), we

247   accessed human and mouse RNA-seq data from 13 tissues (6 neural and 7 non-neural) from the DRG

248   sensoryomics repository[75]. The data contain relative gene abundances in standardized transcripts per

249   million mapped reads and have been normalized to allow comparison across genes. The proportions of

250   gene expression in the CNS (neural proportion score) and DRG (DRG enrichment score) in the context of

251   profiled tissues were calculated, as described in Ray et al[75]. Scores ranging from 0 to 1 were used to

252   denote the strength of tissue enrichment.

253   **Drug repurposing**

254   We examined the drug repurposing status of genes in EAs (N = 156) with high causal probability

255   from fine mapping and transcriptomic and proteomic analyses, using the Druggable Genome database[76].

256   For completeness, we also included the significantly associated genes mapped to GWS variants and

257   MAGMA results in AAs (N = 7) and HAs (N = 2). The Druggable Genome database contains 4,479 coding

258   gene sets with the potential to be modulated by a drug-like small molecule based on their nucleotide

259   sequence and structural similarity to targets of existing drugs[76]. This druggable genome was divided into

260   three tiers. Tier 1 (N = 1,427) contains targets of licensed small molecules and biotherapeutic drugs

261   (curated from the ChEMBL database[77]) and drugs in clinical development. Tier 2 (N = 682) includes

262   targets with verified bioactive drug-like small molecule binding partners and > 50% identity with

263   approved drug targets based on their nucleotide sequence. Tier 3 (N = 2,370) comprises targets or

264   secreted proteins with more distant similarity with an approved drug and members of active protein

265   complexes not included in Tiers 1 and 2. All causal genes and those reported in any of the three tiers of

266   the Druggable Genome were also examined for interaction with prescription drug targets in clinical

267   development using the Drug-Gene Interaction database (DGIdb)[78], which compiles clinical trial

268   information from the FDA, PharmGKB, Therapeutic Target Database, and DrugBank databases, among

269   others. We categorized each prescription drug identified using the Anatomical Therapeutic Chemical

270   classification system, retrieved from the Kyoto Encyclopedia of Genes and Genomics

271   (https://www.genome.jp/kegg/drug/).

Genetic correlation

273   We used LDSC[56] to calculate the r_g of pain intensity with (a) 89 other published pain, substance

274   use, medication use, psychiatric, and anthropometric traits from EA datasets selected using prior

275   epidemiological evidence and (b) 12 psychiatric, substance use, and anthropometric traits based on

276   available AA GWAS summary data (see Supplementary Tables 24 and 26 for detailed lists). In EAs, all

277   traits were tested using pre-computed LD scores for HapMap3[57], while in AAs, LD scores derived using

278   cov-LDSC[58] from MVP AA genotype data were used. In a hypothesis-neutral manner, we also calculated

279   r_gs of pain intensity with 1344 published and unpublished traits from the UKBB using the Complex Trait

280   Virtual Lab (CTG-VL) (https://genoma.io/). CTG-VL is a free open-source platform that incorporates

281   publicly available GWAS data that allow for the calculation of r_g for complex traits using LDSC[79]. Each set

282   of r_g analyses was Bonferroni corrected to control for multiple comparisons (α = 0.05/number of traits

283   tested).

284   We also estimated the cross-ancestry r_gs for pain intensity between AAs, EAs and HAs using

285   Popcorn[80], a computational method that determines the correlation of causal-variant effect sizes at

286   SNPs common across population groups using GWAS summary-level data and LD information. Ancestry-

287   specific LD scores were derived from the 1000 Genomes reference population[48].

Polygenic risk score-based phenome-wide association studies

289   We calculated polygenic risk scores (PRS) for pain intensity and performed a PheWAS analysis in

290   two samples – the Yale-Penn sample and the Penn Medicine Biobank (PMBB). The Yale-Penn sample[81]

291   was deeply phenotyped using the Semi-Structured Assessment for Drug Dependence and Alcoholism

292   (SSADDA), a comprehensive psychiatric instrument that assesses physical, psychosocial, and psychiatric

293   aspects of SUDs and comorbid psychiatric traits[82,83]. As described in detail previously[81], genotyping was

294   performed using the Illumina HumanOmni1-Quad microarray, the Illumina HumanCoreExome array, or

295   the Illumina Multi-Ethnic Global array, followed by imputation using Minimac3[84] and the 1000 Genomes

296   Project phase3 reference panel[48] implemented on the Michigan imputation server

297   (https://imputationserver.sph.umich.edu). SNPs with imputation quality (INFO) score <0.7, MAF <

298   0.01, missingness > 0.01, or an allele frequency difference between batches > 0.04; and individuals with

299   genotype call rate <0.95, or related individuals with pi-hat > 0.25 were excluded. PCs were used to

300   determine genetic ancestry based on the 1000 Genomes Project phase3[48]. The resulting dataset

301   included 4,922 AAs and 5,709 EAs.

302   The PMBB[85] is linked to EHR phenotypes. PMBB samples were genotyped with the GSA

303   genotyping array. Genotype phasing was done using EAGLE[84] and imputation was performed using

304   Minimac3[84] on the TOPMed Imputation server[47]. Following QC (INFO< 0.3, missingness >0.95, MAF > 0.5,

305   sample call rate > 0.9), PLINK 1.90 was used to identify and remove related individuals based on identity

306   by descent (Pi-hat > 0.25). To estimate genetic ancestry, PCs were calculated using SNPs common to the

307   PMBB and the 1000 Genomes Project phase3[48] and the smartpca module of the Eigensoft package

308   (https://github.com/DReichLab/EIG). Participants were assigned to an ancestral group based on the

309   distance of 10 PCs from the 1000 Genomes reference populations. The resulting dataset included 10,383

310   AAs and 29,355 EAs.

311   PRSs for pain intensity were calculated in the Yale-Penn and the PMBB datasets using PRS-

312   Continuous shrinkage software (PRS-CS)[86], with the default setting used to estimate the shrinkage

313   parameters and the random seed fixed to 1 for reproducibility. To identify associations between the

314   pain intensity PRSs and phenotypes, we performed a PheWAS in each dataset by fitting logistic

315   regression models for binary traits and linear regression models for continuous traits. Analyses were

316   conducted using the PheWAS v0.12 R package[87] with adjustment for sex, age at enrollment (in PMBB) or

317   at interview (in Yale-Penn) and the first 10 PCs within each genetic ancestry. We Bonferroni corrected

318   each ancestry-specific analysis (Yale-Penn EAs and AAs: $P < 8.10 \times 10^{-5}$, PMBB EAs and AAs: $P < 3.68 \times 10^{-5}$

319   ).

320   **Mendelian Randomization**

321   We used two-sample Mendelian randomization[88] to evaluate causal associations between 16

322   genetically correlated traits and pain intensity among EAs only because the two other population groups

323   provided inadequate statistical power for the analysis. We inferred causality bidirectionally using three

324   methods: weighted median, inverse-variance weighted (IVW) and MR-Egger, followed by a pleiotropy

325   test using the MR Egger intercept. Instrumental variants were associated with the exposure

326   at $P < 1 \times 10^{-5}$ and a clumping threshold of $r^2 = 0.01$. Potential causal effects were those for which at

327   least two MR tests were significant after multiple correction ($P = 3.13 \times 10^{-3}$, 0.05/16) and did not

328   violate the assumption of horizontal pleiotropy (MR-Egger intercept $P > 0.05$).

329   **Results**

330   **Description of the sample**

331   The study sample comprised 598,339 individuals (AA = 112,968, EA = 436,683, HA = 48,688), of

332   whom 91.2% were male (Supplementary Table 1). The supplementary analyses from which individuals

333   with a lifetime OUD diagnosis were excluded were reduced by 5% across population groups (AA =

334   104,050, EA = 415,740, HA = 46,169) (Supplementary Table 1). The median ages were 61.4 (s.d = 14.0)

335   and 61.7 (s.d = 14.1) in the full and supplementary samples, respectively. About half of individuals in

336   both the full sample (51.2%) and the supplementary sample (52.7%) reported a median NRS of 0, i.e., no

337   pain. Mild (NRS 1-3), moderate (NRS 4-6) and severe pain (NRS 7-10) were reported by 24.4%, 19.2%,

338   and 4.5%, respectively in the full sample, and 24.6%, 18.2%, and 4.0%, respectively in the supplementary

339   sample.

340   **Identification of pain intensity risk loci**

341   In our cross-ancestry meta-analysis of AA, EA, and HA samples, we identified 4,416 GWS variants

342   represented by 158 LD-clumped index variants ($r^2 > 0.1$) (Figure 1). Analyses conditioned on the lead

343   SNP left 125 independent association signals (Supplementary Table 2), 42 of which have previously been

344   reported as pain-related loci[23,25] and 82 of which are novel (Supplementary Table 2). Eight independent

345   variants are exonic, 84 reside within a gene transcript, and 33 are intergenic. Of the 8 exonic variants, 2

346   have likely damaging (PolyPhen > 0.5, CADD > 15) effects (*SLC39A8*-rs13107325 and *WSCD2*-rs3764002)

347   and 5 are potentially deleterious (CADD > 15; *ANAPC4*-rs34811474, *MIER*-rs2034244, *NUCB2*-rs757081,

348   *AKAP10*-rs203462 and *APOE*-rs429358) (Supplementary Table 2). The GWAS in EAs yielded 103 LD

349   clumps ($r^2 > 0.1$) across 86 independent loci (Supplementary Figure 2, Supplementary Table 3). Of these,

350   15 were not GWS in the cross-ancestry meta-analysis (Supplementary Table 3). We also identified 2

351   GWS variants in 1 locus (nearest gene *PPARD*; chr 6) in AAs, and 15 GWS variants in 2 loci (nearest genes

352   *RNU6-461P*; chr 3 and *RNU6-741P*; chr 15) in HAs (Supplementary Table 4).

353   [Insert Figure 1 here]

354   We used a sign test to examine the 86 independent EA index variants in AAs and HAs, of which

355   57 and 74, respectively, were directly analyzed or had proxy SNPs in these populations (Supplementary

356   Table 5). Most variants had the same direction of effect in both populations (N_{SNPs} AAs = 41, HAs = 61;

357   sign test AAs P = .0013, HAs P = 1.39 × 10^{-8}). Only 15 variants (N_{SNPs} AAs = 2, HAs = 13) were nominally

358   associated (P < 0.05) and none survived multiple test correction (Supplementary Table 5). The cross-

359   ancestry genetic-effect correlation (ρ_{pe}) was 0.71 (SE = 0.13, P = 2.12 × 10^{-2}) between EAs and AAs and

360   0.74 (SE = 0.08, P = 6.81 × 10^{-4}) between EAs and HAs. The cross-ancestry heritability estimates between

361   AAs and HAs were too low to calculate ρ_{pe} between those ancestries.

362   In the supplementary analysis that excluded participants with a lifetime OUD diagnosis, we

363   identified 3,400 SNPs in 101 LD-independent risk loci (Supplementary Table 6). Of these, 87 were GWS,

364   13 were $p < 10^{-6}$ in the primary GWAS, and 18 were ancestry specific (17 in EAs and 1 in AAs)

365   (Supplementary Tables 7 & 8).

Single-nucleotide polymorphism heritability and enrichment

367   The proportion of variation in pain intensity explained by common genetic variants (h^2_{SNP}) was

368   similar both for the full samples (AAs: 0.06 ± 0.009 and EAs: 0.08 ± 0.003) and the supplementary

369   samples without OUD (AAs: 0.07 ± 0.009 and EAs: 0.08 ± 0.003) (Supplementary Table 9).

370   Partitioning the SNP heritability for pain intensity revealed significant tissue-group enrichment

371   in central nervous system (CNS) (P = 1.47 × 10^{-12}), adrenal (P = 8.97 × 10^{-5}), liver

372   (P = 3.15 × 10^{-4}), skeletal (P = 8.50 × 10^{-4}) and cardiovascular (P = .001) tissues (Figure 2A & B,

373   Supplementary Table 10). In gene expression datasets derived from multiple tissues, we observed

374   predominant h^2_{SNP} effects in brain (P = 2.87 × 10^{-5}), including hippocampus (P = 1.00 × 10^{-4}) and

375   limbic system (P = 1.15 × 10^{-4}) (Figures 2C & D, Supplementary Table 11). SNP-based heritability in

376   histone modification data also showed robust enhancer (H3K27ac and H3K4me1) and active promoter

377   (H3K4me3 and H3K9ac) enrichments in brain tissues, including the dorsolateral prefrontal cortex

378   (P < 1.32 × 10^{-4}), inferior temporal lobe (P < 3.09 × 10^{-4}), angular gyrus (P = 8.42 × 10^{-5}), and

379   anterior caudate (P = 1.12 × 10^{-4}) (Figure 2E, Supplementary Table 12). Similar results were obtained

380   for the partitioned heritability analysis of the supplementary GWAS (Supplementary Tables 11 & 12),

381   though it also included significant expression effects in the cortex and cerebellum.

382   Although the SNP-based heritability and enrichment for the full and supplementary GWASs

383   were similar, because the full sample yielded more risk loci, we based all downstream analyses (except

384   genetic correlation [r_g] analyses) on the GWAS results from that sample.

385   [Insert Figure 2 here]

386   **Gene-set enrichment in tissue and cell types**

387   To clarify the potential transcriptomic mechanism of each GWS pain locus, we mapped GWAS

388   variants to genes via expression quantitative trait locus (eQTL) association in GTEx[61] and assessed the

389   tissue enrichment of mapped genes in FUMA[52]. After correcting for multiple testing ($P = 9.25 \times 10^{-4}$)

390   in the cross-ancestry and EA-specific GWASs, we uncovered significant transcriptomic enrichment only

391   in brain tissues (Supplementary Figure 3). Consistent with previous findings of brain tissue enrichment

392   across different pain phenotypes in EAs[22,25,27], both our EA and cross-ancestry analyses showed notable

393   enrichment in the cerebellum (cross-ancestry, $P = 2.48 \times 10^{-7}$; EA, $P = 2.90 \times 10^{-6}$), cerebellar

394   hemisphere (cross-ancestry, $P = 4 \times 10^{-7}$; EA, $P = 6.23 \times 10^{-6}$), cortex (cross-ancestry,

395     $P = 2.79 \times 10^{-6}$; EA, $P = 3 \times 10^{-4}$), and frontal cortex (cross-ancestry, $P = 2.82 \times 10^{-6}$; EA,

396     $P = 4.17 \times 10^{-4}$) (Supplementary Figure 3). Among AAs there were no significantly enriched tissues

397   (Supplementary Table 13).

398   To investigate enrichment at the level of the cell type in the EA GWAS results, we conducted

399   FUMA cell-type specific analysis[67] in a collection of cell types in 13 human brain sc-RNAseq datasets.

400   After adjusting for possible confounding due to correlated expression within datasets using a stepwise

401   conditional analysis, we detected jointly significant cell-type enrichments (proportional significance, PS >

402   0.5) for GABAergic neurons largely in the human adult mid-brain ($P = 0.003$ $\beta = 0.206$, s.e. = 0.075, PS

403   0.56) and to a lesser extent in the prefrontal cortex ($P = 0.044$, $\beta = 0.045$, s.e. = 0.016, PS 0.39)

404   (Supplementary Table 14).

405   **Prioritization of candidate genes**

406   To facilitate the biological interpretation and identification of druggable targets, we used a

407   combination of MAGMA and fine-mapping, transcriptomic, proteomic, and chromatin interaction

408   models to prioritize high-confidence variants and genes that most likely drive GWAS associations.

409   Assigning SNPs to genes using physical proximity, MAGMA gene-based analyses[63] identified 6 GWS

410   genes in AAs, 203 in EAs, and 125 in the cross-ancestry results (Supplementary Figure 4, Supplementary

411   Table 15), but none in HAs. MAGMA gene-set analysis[63] using cross-ancestry GWAS results identified

412   significantly enriched biological processes in catecholamine uptake (GO:0051944; Bonferroni P=0.019)

413   and startle response (GO:0001964; Bonferroni P=0.024). Negative regulation of synaptic transmission

414   (GO:0050805; Bonferroni P=0.016) was related to pain intensity in EAs (Supplementary Table 16).

415   For consistency with available reference data, we based the fine mapping procedure on EA

416   GWAS results using 78 genomic regions (spanning 103 index variants) (Supplementary Table 17) defined

417   by the maximum physical distance between the LD block of independent lead SNPs (Methods).

418   Functional genomic prediction models used the full EA GWAS results (Supplementary Figure 1).

419   We fine-mapped the 78 regions using the Bayesian method implemented in FINEMAP[54]

420   (Methods). For each region with independent causal signals (Supplementary Table 17), credible sets of

421   variants (PP > 0.5) were constructed to capture 95% of the regional posterior probability (k ≤ 5,

422   Supplementary Table 18). Of these regions, 4 harbored 1 SNP (potentially indicating the causal variant),

423   20 regions 2 SNPs and 44 regions 3 or more SNPs (Supplementary Table 18). In total, FINEMAP

424   prioritized 76 unique credible variants (N = 108, Figure 3A), including 26 independent lead SNPs and 18

425   novel pain loci (Figure 3B). Most (50/76) of the credible variants map to protein-coding genes and are

426   mostly eQTLs (Supplementary Table 18), and five harbor missense variants, of which three (*ANAPC4,*

427   *APOE,* and *SLC39A8*) are known pain loci[25,31] and two (*RYR2* and *AKAP10*) are novel (Figure 3B). This

428   small proportion of missense variants and high eQTL enrichment are consistent with an increased

429   probability that the credible variants influence liability to pain intensity through gene expression

430   modulation.

431   We performed TWAS and PWAS analyses to determine whether risk variants exert their effects

432   via gene and/or protein expression. After correction for multiple testing, 196 unique genes (TWAS eQTL

433   – 294, TWAS sQTL – 67 and PWAS – 32) were associated with pain intensity (Supplementary Tables 19 &

434   20). Of these, 69 represent novel associations (based on a window from the index GWAS locus > 1 MB).

435   PWAS showed significant associations in the dorsolateral prefrontal cortex (dlPFC) that overlapped for

436   22 unique genes across multiple brain tissues in the TWAS (eQTL – 16, sQTL – 8) (Figure 3C).

437   [Insert Figure 3 here]

438   Chromatin interaction mapping using Hi-C data in adult and fetal brain identified 512 unique

439   significantly interacting genes (P=2.84$\times$10^{-8}) (Supplementary Table 21), of which 60 are associated

440   with all six chromatin annotations (Supplementary Figure 5) and 20 overlap with TWAS and/or PWAS

441 findings, including *DPYSL5, KHK, MAPRE3, MST1R, NEK4, GNL3, GRK4, UHRF1BP1* and *VKORC1* (Figure

442 3C, Supplementary Tables 19, 20 & 21).

443 Based on concordant evidence from colocalization analyses in TWAS and PWAS (COLOC PP4 >

444 0.80), 104 unique genes (TWAS eQTL – 139, TWAS sQTL – 20 and PWAS – 14) were putatively causal for

445 pain intensity (Supplementary Tables 19 & 20), of which 10 (including *DPYSL5, GRK4, KHK* and *MST1R*)

446 were validated by SMR analysis (P_{HEIDI} > 0.05) (Figure 3D, Supplementary Table 22). Among the 104

447 genes, 6 (*CHMP1A, GRIA1, GRK4, MST1R, STMN3* and *TRAF3*) captured 50% or more of the FINEMAP

448 posterior probability (Supplementary Table 18). Notably, the *MST1R* intronic locus (rs9815930), which is

449 in a credible set that harbors four other variants in high LD with the novel index variant rs2247036

450 (nearest gene – *TRAIP*) (Supplementary Figure 6), displayed the most robust causal effects from COLOC

451 and SMR in more than one brain tissue (Figure 3D).

452 We also explored enrichment of causal genes and proteins in the dorsal root ganglia (DRG),

453 which are important for transduction of nociceptive signals from the periphery to the CNS. None of the

454 causal genes or proteins (N = 104) were enriched in human or mouse DRG (DRG enrichment score > 0.5)

455 (Supplementary Figure 7A). Supporting results from TWAS and PWAS, 63 unique genes (human – 38 and

456 mouse – 49) were primarily enriched in the CNS, of which 22 (including *GRK4, GRIA1, MAPRE3, NEK4,*

457 *STMN3* and *TRAF3*) showed common enrichment patterns across species (Supplementary Figure 7B).

458 Integrating FINEMAP, colocalization and SMR prioritized 156 high-confidence genes underlying

459 the pain intensity GWAS association, of which 5 are exonic and missense (Supplementary Table 23), and

460 151 exert their effect via gene or protein expression.

461 **Phenotypic correlates of pain intensity**

462 As expected, the strongest positive genetic correlations of pain intensity were with other pain

463 phenotypes (e.g., multisite chronic pain r_g=0.789, osteoarthritis r_g=0.710, neck/shoulder pain r_g=0.669,

464 back pain r_g=0.697, hip pain r_g=0.729, knee pain r_g=0.637; Figure 4A). Of 72 medical, anthropometric, or

465 psychiatric traits associated epidemiologically with pain severity and mortality, 56 were significantly

466 genetically correlated with pain intensity in EAs (Bonferroni $P < 5.62 \times 10^{-4}$) (Figure 4A, Supplementary

467 Table 24).

468 [Insert Figure 4 here]

469   Notably, the liability to pain intensity was significantly positively genetically correlated with

470   neuroticism, depression, insomnia, a variety of smoking-related measures, cannabis use disorder (CUD),

471   alcohol dependence, OUD, and overweight and obesity (Figure 4A). As in prior studies[24,29,89], pain

472   intensity was significantly negatively correlated with educational attainment, cognitive performance,

473   intelligence, and age of smoking initiation (Figure 4A). Relevant to drug repurposing, pain intensity was

474   also positively correlated with the use of a variety of analgesic and anti-inflammatory drugs (Figure 4A).

475   We also found significant r_gs with pain intensity for several medical conditions and health outcomes in

476   the UKBB (including genitourinary disease, chronic bronchitis, angina, etc., Bonferroni $P < 3.72 \times 10^{-5}$,

477   Supplementary Table 25). In AAs, pain intensity was positively genetically correlated with PTSD-related

478   features (e.g., re-experiencing, hyperarousal) and nominally associated ($p < 0.05$) with substance use

479   traits (e.g., maximum alcohol intake and smoking trajectory, Supplementary Table 26).

480   In the Yale-Penn sample, we calculated PRS for 4,922 AAs and 5,709 EAs. Among AAs, none of

481   the associations survived Bonferroni correction, likely due to the smaller discovery sample than for EAs

482   (Supplementary Figure 8, Supplementary Table 27). In EAs, PheWAS identified 147 phenotypes,

483   including 107 in the substance-related domain (40 opioid-related, 30 cocaine-related, 20 tobacco-

484   related, 12 alcohol-related, and 6 cannabis-related) and 39 in other domains (9 medical, 18 psychiatric

485   [9 PTSD, 5 ADHD, 2 conduct disorder, and 2 antisocial], 7 early childhood environmental, and 5

486   demographic phenotypes) that were significantly associated with the pain PRS (Supplementary Figure 9,

487   Supplementary Table 27). The most significant findings were a negative association of the pain severity

488   PRS with educational attainment ($P = 2.39 \times 10^{-26}$) and a positive association with the Fagerström Test

489   for Nicotine Dependence ($P = 4.71 \times 10^{-25}$). Opioid dependence was also positively associated with the

490   pain PRS ($P = 3.87 \times 10^{-12}$), and remained significant when using a PRS based on the supplementary

491   GWAS that excluded individuals with an OUD diagnosis (OR = 1.27, $P = 1.35 \times 10^{-6}$).

492   In PMBB, we calculated PRS for 10,383 AAs and 29,355 EAs. In AAs, no association with the pain

493   PRS survived Bonferroni correction (Supplementary Figure 10, Supplementary Table 28). In EAs, the pain

494   severity PRS was associated with 63 phenotypes, including 7 pain phenotypes and 6 psychiatric

495   disorders (i.e., substance-, depression-, and anxiety-related traits). Other phenotypic categories with

496   associations with the pain severity PRS were circulatory system (n=11), infectious diseases (n=4),

497   endocrine/metabolic (n=8), genitourinary (n=2), musculoskeletal (n=3), and neoplasms (n=4). The most

498   significant findings were positive correlations with obesity ($P = 1.97 \times 10^{-45}$) and tobacco use disorder

499　　($P = 1.55 \times 10^{-24}$) and a negative association with benign neoplasm of skin ($P = 2.67 \times 10^{-26}$)

500　　(Supplementary Figure 11, Supplementary Table 28).

501　　　　　Two-sample MR between genetically correlated traits (N = 16) and pain intensity yielded 10

502　　traits with evidence of causal association, 8 of which were bidirectional (Supplementary Table 29).

503　　Genetically predicted higher opioid use (N02A), depressed affect subcluster, major depressive disorder,

504　　neuroticism, use of drugs to treat peptic ulcer, and smoking cessation (coded as current smoking) had a

505　　significant positive bidirectional causal effect with pain intensity, whereas educational attainment and

506　　cognitive performance had a significant negative bidirectional causal effect (Supplementary Table 29).

507　　Further, increased risk of pain intensity positively predicted smoking initiation and cigarettes per day.

508　　**Genetically inferred drug repurposing**

509　　　　　Of the 156 genes in EAs with evidence supporting causality from fine-mapping and functional

510　　genomic prediction, 20 were present in the druggable genome database[76] (Supplementary Table 30). Of

511　　these druggable candidate genes, 11 (including *GRIA1*, *GRK4* and *MST1R*) are tier-1 candidates, which

512　　includes targets of licensed drugs and drugs in clinical trial, 4 genes (e.g., *NEK4* and *RYR2*) are in tier 2,

513　　and 4 are in tier 3 (Supplementary Table 30). Within tier 1, drugs that interact with *GRK4* (a credible pain

514　　gene locus in moderate LD with the novel index variant *NOP14**rs71597204 – Supplementary Figure 12)

515　　are beta-blockers (atenolol and metoprolol) and a calcium-channel blocking agent (verapamil) (Figure

516　　4B), which have analgesic effects in osteoarthritis[90,91] and migraine[92]. Another tier-1 candidate gene –

517　　*GRIA1* – is targeted by anesthetics (sevoflurane, isoflurane, desflurane), antiepileptics (topiramate,

518　　perampanel), analgesics (methoxyflurane), psychoanaleptics (piracetam, aniracetam), and a diuretic

519　　(cyclothiazide) (Figure 4B). Drug classes for pain intensity also included anti-hemorrhagic agents (e.g.,

520　　fostamatinib [tier 1: *MST1R* and *FYN*; tier 2: *NEK4*] and menadione [*VKORC1*]) (Figure 4B, Supplementary

521　　Table 30).

522　　　　　Of the 7 genes associated with pain intensity in AAs, *PPARD*, which harbors the new genetic

523　　signal discovered in this study, is a tier-1 druggable candidate with 30 interacting drug classes

524　　(Supplementary Table 30). The *PPARD* negative modulator sulindac is an approved non-steroidal anti-

525　　inflammatory and antirheumatic drug used to treat osteoarthritis.

526

527　　**Discussion**

528   We conducted the largest multi-ancestry, single-sample GWAS of pain intensity to date,

529 comprising 112,968 AA, 436,683 EA, and 48,688 HA individuals. Cross-ancestry analyses identified 125

530 independent risk loci, of which 82 have not previously been associated with any pain phenotype.

531 Although prior GWASs for chronic pain phenotypes have identified 99 loci[23–27,32], the study samples have

532 largely been limited to EA individuals. The diversity of the MVP sample enabled us to identify novel

533 association signals in both AAs (*PPARD**rs9470000) and HAs (nearest genes *RNU6-461P**rs146862033,

534 *RNU6-741P**rs1019597899).

535   Findings from gene set analysis, tissue enrichment, and cell-type specificity highlight novel

536 biological pathways linking genetic variation to the etiopathology of pain. These functional analyses all

537 implicate the brain, providing genetic support to the current understanding of the pathophysiology of

538 pain severity[93]. Genes predominantly expressed in the CNS, particularly in the cerebellum, cerebellar

539 hemisphere, and cortex region, rather than in the DRG, appear to play a salient role in modulating the

540 intensity of pain, consistent with prior associations of sustained chronic pain intensity with increased

541 activity in these brain regions[94–96]. Our findings are also consistent with prior reports[24,25,97,98] of enriched

542 gene expression in brain that contribute to pain intensity in a dose- and time-dependent manner and

543 may involve specific neuronal processes in brain regions implicated in emotional processing[93]. Evidence

544 that GABAergic neurons are cells of specific interest is a key novel finding. GABA has long been

545 implicated in the modulation and perception of pain[99–101] and previous work has implicated specific

546 GABAergic activity in the midbrain as a modulator of pain and anxiety[102]. Altered GABA levels have been

547 reported in individuals with various types of pain[103,104], and have been associated with greater self-

548 reported pain[105]. Targeting GABA functioning (e.g.,[106]), particularly in the brain regions enriched for pain

549 intensity, may represent a novel therapeutic strategy.

550   Eleven of 156 prioritized genes encode druggable small molecules that are targets of licensed

551 drugs or those in clinical trials, representing drug repurposing opportunities for treating chronic pain.

552 We highlight *GRK4* and *GRIA1*, each with at least three lines of evidence supporting their involvement in

553 chronic pain. *GRK4* encodes G protein-coupled receptor kinase 4 and has been linked with

554 hypertension[107], which is associated with chronic pain at the population level[108,109]. Of note, *GRK4*

555 showed significant upregulation in the cerebellar hemisphere, fine maps to an intronic variant with >

556 95% PP, and is a target of beta-blockers. The use of beta-blockers has been associated with reduced

557 osteoarthritis pain scores, prescription analgesic use[90] and consultations for knee osteoarthritis, knee

558 pain, and hip pain[91]. *GRIA1* encodes an ionotropic glutamate receptor subunit, an excitatory

559   neurotransmitter receptor at many synapses in the CNS. Loss-of-function mutations in *GRIA1* are linked

560   to neurodevelopmental impairments[110,111]. The *GRIA1* antagonist sevoflurane reduced pain in patients

561   suffering from chronic venous ulcer[112]. However, clinical trials of topiramate (another drug target for

562   *GRIA1*) for treating neuropathic chronic pain are inconclusive[113]. Research on the mechanisms that

563   underlie the biology of these potential drug targets for *GRK4* and *GRIA1* and their effects on the onset

564   and severity of chronic pain are warranted.

565   Pain intensity was strongly genetically correlated with other chronic pain phenotypes.

566   Corroborating existing epidemiological studies on the comorbid nature of different pain conditions[33], the

567   strongest genetic correlations of pain intensity were with multisite chronic pain, followed by pain in

568   specific bodily locations. In line with previous observations in GWASs of other pain-related

569   phenotypes[24,25,27,28,89], there were also positive genetic correlations of pain intensity with psychiatric

570   disorders, substance use and use disorders, and anthropometric traits.

571   PheWAS findings in both the Yale-Penn sample – enriched for individuals with substance-related

572   traits – and the PMBB – comprising a medical population – were prominent in EAs. These findings

573   underscore the important influence of co-occurring substance-related, psychiatric, and medical

574   pathology and educational achievement on the intensity of the pain experience. In contrast, the PRS

575   generated from the pain intensity discovery sample in AAs yielded few associations in either of the

576   target samples, which underscores the need for larger non-European samples to elucidate the genetic

577   architecture of pain intensity.

578   Two-sample MR analysis supported causal associations between pain and multiple traits.

579   Smoking has previously been associated with greater pain intensity, but studies can be confounded by

580   socioeconomic factors, and a bi-directional relationship has been proposed[114]. Here, we show evidence

581   for a causal relationship of pain on the number of cigarettes smoked per day, smoking initiation, and

582   smoking cessation. In line with previous findings[25,33,35,36], pain intensity had a bidirectional causal effect

583   on the risk of both depression and neuroticism, suggesting that greater pain could predispose

584   individuals to increased risk for these psychiatric disorders and vice versa. Supporting the positive

585   genetic correlation between opioid use and pain intensity, MR showed evidence of a bidirectional causal

586   effect between pain intensity and opioid use.

587   Our findings underscore the complex nature of pain intensity, with the hundreds of genetic loci

588   contributing to the experience of pain identified here and in prior studies reflecting a substantial genetic

589 contribution to pain-related traits. The evidence adduced here of pleiotropy of pain intensity with

590 psychiatric traits such as neuroticism and depression reflects the contribution of non-physical factors to

591 the experience of pain intensity. This is consistent with the observed significant tissue-group enrichment

592 in CNS, the predominant gene expression findings in brain (including the hippocampus and limbic

593 system), and the SNP-based enhancer enrichments in histone modification in brain tissues (including the

594 dorsolateral prefrontal cortex, inferior temporal lobe, angular gyrus, and anterior caudate).

595 A limitation of the present study concerns the NRS phenotype. Although such a quantitative trait

596 is more informative than a binary one (e.g., the presence of a specific pain diagnosis), it is based on

597 subjective report. However, because the subjective experience of pain is a key defining feature of the

598 clinical phenomenon[1,115] the phenotype has high public health significance. Pain scores recorded by

599 clerks and nurses in the clinical setting may consistently under report the patient's response. In earlier

600 work that compared self-reported pain from a direct patient survey to scores recorded in a VA clinical

601 setting[117], we found that, despite lower scores recorded in the clinic the two reports correlated well.

602 Nonetheless, the imprecise measurement of pain intensity likely yields lower power for gene discovery.

603 The routine assessment of pain severity provided a very large number of pain scores, which we reduced

604 by taking the median of medians for each individual as a trait for GWAS. In subsequent analyses, we plan

605 to evaluate alternative methods for characterizing pain severity (e.g., pain trajectories). Another

606 limitation is that our sample comprises predominantly male veterans, which in view of well

607 demonstrated sex differences in the experience and frequency of pain[26], limits the application of the

608 findings to the general population. Finally, although our sample was more diverse than prior GWAS of

609 pain traits, analyses in the AA and HA samples were underpowered.

610 Despite these limitations, the large MVP sample and informative quantitative trait measured

611 repeatedly within subjects enabled us to generate a proxy for chronic pain and identify many novel loci

612 contributing to the trait. Downstream analyses localize the genetic effects largely to four CNS regions

613 and using available single-cell RNAseq data specifically to GABAergic neurons. Combined with drug

614 repurposing findings that implicate 20 druggable targets, the study provides a basis for studies of novel,

615 non-opioid medications for use in alleviating chronic pain.

616

617

618

619   Figure Legends

620   **Figure 1. Manhattan plot for the pain intensity cross-ancestry GWAS meta-analysis.** This identified 125

621   independent index variants. SNPs above the red line are GWS after correction for multiple testing

622   ($P < 5 \times 10^{-8}$)

623

624   **Figure 2. Enrichment of pain intensity in the brain. A,** Partitioning heritability enrichment analyses using

625   LDSC showing enrichment for pain intensity in the CNS, adrenal, liver, cardiovascular, and skeletal

626   tissues. The dashed black lines indicate Bonferroni-corrected significance ($P < 0.005$). **B,** Proportion of

627   heritability shows robust enrichment for SNPs in brain and immune-related tissues. Heritability

628   enrichment analyses for gene expression (**C & D**) and chromatin interaction (top 35 annotations are

629   shown in **E,** see supplementary Table 12 for full details) using GTEx data show enrichment for pain

630   intensity in brain regions previously associated with chronic pain. Bonferroni correction was applied

631   within each tissue conditioned on the number of genes tested.

632

633   **Figure 3. Gene prioritization for pain intensity. A,** Genomic annotation of credible sets using FINEMAP

634   shows enrichment largely in non-coding regions and to a lesser extent in exons. **B,** Annotation of known

635   and novel credible genes. Dashed lines indicate posterior probability > 0.5. **C,** Number of overlapping

636   genes across functional prediction models. **D,** Tissue enrichment of prioritized genes using SMR and

637   GTEx data show enrichment in brain regions. Size of circle reflects $-\log_{10}P$. Bonferroni correction was

638   applied within each tissue conditioned on the number of genes tested.

639

640   **Figure 4. Genetic correlation and drug repurposing. A,** Genetic correlation for pain intensity using LDSC.

641   All points passing Bonferroni correction (Bonferroni correction threshold = 5.62×10^{-4} [0.05/89]) are

642   plotted. The color of the circle indicates the phenotypic category. **B,** Druggable targets and drug

643   interactions for 8 credible genes associated with pain intensity. For a full list of credible drug targets see

644   Supplementary Table 30.

645

646

647 **Data Availability.** The full summary statistics from the meta-analyses will be available through dbGaP

648 upon publication.

649

650 **Code Availability.** Imputation was performed using Minimac3

651 (https://genome.sph.umich.edu/wiki/Minimac3). GWAS was performed using PLINK2 (https://www.cog-

652 genomics.org/plink2). Meta-analyses were performed using METAL

653 (https://genome.sph.umich.edu/wiki/METAL_Documentation). GCTA-COJO

654 (https://cnsgenomics.com/software/gcta/#Overview) was used for identification of independent loci.

655 FUMA (https://fuma.ctglab.nl/) was used for gene association, functional enrichment and gene-set

656 enrichment analyses. Transcriptomic and proteomic analyses were performed using FUSION

657 (https://github.com/gusevlab/fusion_twas). Chromatin accessibility analyses were performed using H-

658 MAGMA (https://github.com/thewonlab/H-MAGMA). LDSC (https://github.com/bulik/ldsc) was used for

659 heritability estimation, genetic correlation analysis (also using the CTG-VL; https://genoma.io) and

660 heritability enrichment analyses. Trans-ancestry genetic correlation was estimated using Popcorn

661 (https://github.com/brielin/Popcorn). PRS analyses were performed using PRS-CS

662 (https://github.com/getian107/PRScs). PheWAS analyses were run using the PheWAS R package

663 (https://github.com/PheWAS/PheWAS). The MendelianRandomization R package (https://cran.r-

664 project.org/web/packages/MendelianRandomization/index.html) was used for MR analyses.

665

687   **Contributions**

688   S.T conducted the main analyses and drafted the manuscript. R.V.S conducted phenotype-related

689   analyses. Z.J and H.X conducted downstream analyses. D.S annotated gene findings. M.P.V and K.S

690   helped conduct analyses. R.V.S, Z.J, H.X, D.S, E.H, M.P.V, K.S, K.X, J.G, D.A.J, C.T.R, M.C, E.S, and S.G.W

691   helped to write the manuscript. A.C.J obtained funding to support the project and helped to write the

692   manuscript. R.L.K supervised the analyses and helped to write the manuscript. H.R.K conceived the

693   project, obtained funding to support it, and helped to supervise the analyses and write the manuscript.

694   All authors reviewed and approved the final version of the manuscript

695   **Ethics declarations**

696   HRK is a member of advisory boards for Dicerna Pharmaceuticals, Sophrosyne Pharmaceuticals, Enthion

697   Pharmaceuticals, and Clearmind Medicine; a consultant to Sobrera Pharmaceuticals; the recipient of

698   research funding and medication supplies from Alkermes for an investigator-initiated study; and a

699   member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which

700   was supported in the last three years by Alkermes, Dicerna, Ethypharm, Lundbeck, Mitsubishi, and

701   Otsuka. HRK and JG are named as inventors on PCT patent application #15/878,640 entitled: "Genotype-

702   guided dosing of opioid agonists," filed January 24, 2018. ES is a full-time employee of Regeneron

703   Pharmaceuticals. The other authors have no disclosures to make.

704

705

References

1. Raja SN, Carr DB, Cohen M, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *PAIN*. 2020;161(9). https://journals.lww.com/pain/Fulltext/2020/09000/The_revised_International_Association_for_th e.6.aspx

2. Scher C, Meador L, Van Cleave JH, Reid MC. Moving Beyond Pain as the Fifth Vital Sign and Patient Satisfaction Scores to Improve Pain Care in the 21st Century. *Pain Manag Nurs*. 2018;19(2):125-129. doi:10.1016/j.pmn.2017.10.010

3. Nestler EJ, Waxman SG. Resilience to Stress and Resilience to Pain: Lessons from Molecular Neurobiology and Genetics. *Trends in Molecular Medicine*. 2020;26(10):924-935. doi:10.1016/j.molmed.2020.03.007

4. Yong RJ, Mullins PM, Bhattacharyya N. Prevalence of chronic pain among adults in the United States. *Pain*. 2022;163(2):e328-e332. doi:10.1097/j.pain.0000000000002291

5. Tompkins DA, Hobelmann JG, Compton P. Providing chronic pain management in the "Fifth Vital Sign" Era: Historical and treatment perspectives on a modern-day medical dilemma. *Drug and Alcohol Dependence*. 2017;173:S11-S21. doi:10.1016/j.drugalcdep.2016.12.002

6. Gaskin DJ, Richard P. The economic costs of pain in the United States. *J Pain*. 2012;13(8):715-724. doi:10.1016/j.jpain.2012.03.009

7. James SL, Abate D, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*. 2018;392(10159):1789-1858. doi:10.1016/S0140-6736(18)32279-7

8. Humphreys K, Shover CL, Andrews CM, et al. Responding to the opioid crisis in North America and beyond: recommendations of the Stanford–Lancet Commission. *The Lancet*. 2022;399(10324):555-604. doi:10.1016/S0140-6736(21)02252-2

9. Friedman JR, Hansen H. Evaluation of Increases in Drug Overdose Mortality Rates in the US by Race and Ethnicity Before and During the COVID-19 Pandemic. *JAMA Psychiatry*. 2022;79(4):379-381. doi:10.1001/jamapsychiatry.2022.0004

10. Maher DP, Wong CH, Siah KW, Lo AW. Estimates of Probabilities of Successful Development of Pain Medications: An Analysis of Pharmaceutical Clinical Development Programs from 2000 to 2020. *Anesthesiology*. 2022;137(2):243-251. doi:10.1097/ALN.0000000000004265

11. Ballantyne JC, Shin NS. Efficacy of opioids for chronic pain: a review of the evidence. *Clin J Pain*. 2008;24(6):469-478. doi:10.1097/AJP.0b013e31816b2f26

12. Cheatle MD, Savage SR. Informed consent in opioid therapy: a potential obligation and opportunity. *J Pain Symptom Manage*. 2012;44(1):105-116. doi:10.1016/j.jpainsymman.2011.06.015

741   13. Els C, Jackson TD, Kunyk D, et al. Adverse events associated with medium- and long-term use of
742   opioids for chronic non-cancer pain: an overview of Cochrane Reviews. *Cochrane Database Syst Rev*.
743   2017;10(10):CD012509. doi:10.1002/14651858.CD012509.pub2

744   14. McDonagh M, Selph S, Buckley D. *Nonopioid Pharmacologic Treatments for Chronic Pain*. Rockville
745   (MD): Agency for Healthcare Research and Quality (US); 2020. Available from:
746   https://www.ncbi.nlm.nih.gov/books/NBK556277/

747   15. Nelson MR, Tipney H, Painter JL, et al. The support of human genetic evidence for approved drug
748   indications. *Nature Genetics*. 2015;47(8):856-860. doi:10.1038/ng.3314

749   16. King EA, Davis JW, Degner JF. Are drug targets with genetic support twice as likely to be approved?
750   Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug
751   approval. *PLoS Genet*. 2019;15(12):e1008489. doi:10.1371/journal.pgen.1008489

752   17. Pushpakom S, Iorio F, Eyers PA, et al. Drug repurposing: progress, challenges and recommendations.
753   *Nature Reviews Drug Discovery*. 2019;18(1):41-58. doi:10.1038/nrd.2018.168

754   18. Nielsen C, Knudsen G, Steingrímsdóttir Ó. Twin studies of pain. *Clinical Genetics*. 2012;82(4):331-
755   340. doi:10.1111/j.1399-0004.2012.01938.x

756   19. Sexton JE, Cox JJ, Zhao J, Wood JN. The Genetics of Pain: Implications for Therapeutics. *Annu Rev
757   Pharmacol Toxicol*. 2018;58(1):123-142. doi:10.1146/annurev-pharmtox-010617-052554

758   20. Abboud C, Duveau A, Bouali-Benazzouz R, et al. Animal models of pain: Diversity and benefits.
759   *Journal of Neuroscience Methods*. 2021;348:108997. doi:10.1016/j.jneumeth.2020.108997

760   21. Meng W, Adams MJ, Palmer CNA, et al. Genome-wide association study of knee pain identifies
761   associations with GDF5 and COL27A1 in UK Biobank. *Communications Biology*. 2019;2(1):321.
762   doi:10.1038/s42003-019-0568-2

763   22. Meng W, Chan BW, Harris C, et al. A genome-wide association study finds genetic variants
764   associated with neck or shoulder pain in UK Biobank. *Human Molecular Genetics*. 2020;29(8):1396-
765   1404. doi:10.1093/hmg/ddaa058

766   23. Suri P, Palmer MR, Tsepilov YA, et al. Genome-wide meta-analysis of 158,000 individuals of
767   European ancestry identifies three loci associated with chronic back pain. *PLoS Genet*.
768   2018;14(9):e1007601. doi:10.1371/journal.pgen.1007601

769   24. Freidin MB, Tsepilov YA, Palmer M, et al. Insight into the genetic architecture of back pain and its
770   risk factors from a study of 509,000 individuals. *Pain*. 2019;160(6):1361-1373.
771   doi:10.1097/j.pain.0000000000001514

772   25. Johnston KJA, Adams MJ, Nicholl BI, et al. Genome-wide association study of multisite chronic pain
773   in UK Biobank. *PLOS Genetics*. 2019;15(6):e1008164. doi:10.1371/journal.pgen.1008164

774   26. Johnston KJA, Ward J, Ray PR, et al. Sex-stratified genome-wide association study of multisite
775   chronic pain in UK Biobank. *PLoS Genet*. 2021;17(4):e1009428. doi:10.1371/journal.pgen.1009428

776   27. Mocci E, Ward K, Dorsey SG, Ament SA. GWAS meta-analysis reveals dual neuronal and
777   immunological etiology for pain susceptibility. *medRxiv*. Published online January 1,
778   2021:2021.08.23.21262510. doi:10.1101/2021.08.23.21262510

779   28. Rahman MS, Winsvold BS, Chavez Chavez SO, et al. Genome-wide association study identifies
780   RNF123 locus as associated with chronic widespread musculoskeletal pain. *Ann Rheum Dis*.
781   2021;80(9):1227-1235. doi:10.1136/annrheumdis-2020-219624

782   29. Meng W, Adams MJ, Hebert HL, Deary IJ, McIntosh AM, Smith BH. A Genome-Wide Association
783   Study Finds Genetic Associations with Broadly-Defined Headache in UK Biobank (N=223,773).
784   *EBioMedicine*. 2018;28:180-186. doi:10.1016/j.ebiom.2018.01.023

785   30. Tachmazidou I, Hatzikotoulas K, Southam L, et al. Identification of new therapeutic targets for
786   osteoarthritis through genome-wide analyses of UK Biobank data. *Nature Genetics*. 2019;51(2):230-
787   236. doi:10.1038/s41588-018-0327-1

788   31. Boer CG, Hatzikotoulas K, Southam L, et al. Deciphering osteoarthritis genetics across 826,690
789   individuals from 9 populations. *Cell*. 2021;184(18):4784-4818.e17. doi:10.1016/j.cell.2021.07.038

790   32. Zorina-Lichtenwalter K, Parisien M, Diatchenko L. Genetic studies of human neuropathic pain
791   conditions: a review. *Pain*. 2018;159(3):583-594. doi:10.1097/j.pain.0000000000001099

792   33. Meng W, Adams MJ, Reel P, et al. Genetic correlations between pain phenotypes and depression
793   and neuroticism. *European Journal of Human Genetics*. 2020;28(3):358-366. doi:10.1038/s41431-
794   019-0530-2

795   34. Zorina-Lichtenwalter K, Bango CI, Van Oudenhove L, et al. Identification and characterization of
796   genetic risk shared across 24 chronic pain conditions in the UK Biobank. *medRxiv*. Published online
797   January 1, 2022:2022.06.28.22277025. doi:10.1101/2022.06.28.22277025

798   35. Tang B, Meng W, Hägg S, Burgess S, Jiang X. Reciprocal interaction between depression and pain:
799   results from a comprehensive bidirectional Mendelian randomization study and functional
800   annotation analysis. *Pain*. 2022;163(1):e40-e48. doi:10.1097/j.pain.0000000000002305

801   36. Farrell SF, Kho PF, Lundberg M, et al. A Shared Genetic Signature for Common Chronic Pain
802   Conditions and its Impact on Biopsychosocial Traits. *The Journal of Pain*. Published online October
803   14, 2022. doi:10.1016/j.jpain.2022.10.005

804   37. Troiani V, Crist RC, Doyle GA, et al. Genetics and prescription opioid use (GaPO): study design for
805   consenting a cohort from an existing biobank to identify clinical and genetic factors influencing
806   prescription opioid use and abuse. *BMC Med Genomics*. 2021;14(1):253. doi:10.1186/s12920-021-
807   01100-z

808   38. Gaziano JM, Concato J, Brophy M, et al. Million Veteran Program: A mega-biobank to study genetic
809   influences on health and disease. *Journal of Clinical Epidemiology*. 2016;70:214-223.
810   doi:10.1016/j.jclinepi.2015.09.016

811   39. Luther SL, Finch DK, Bouayad L, et al. Measuring pain care quality in the Veterans Health
812   Administration primary care setting. *PAIN*. 2022;163(6).

813   https://journals.lww.com/pain/Fulltext/2022/06000/Measuring_pain_care_quality_in_the_Veteran
814   s_Health.5.aspx

815   40. Farrar JT. A consideration of differences in pain scales used in clinical trials. *PAIN*. 2022;163(12).
816   https://journals.lww.com/pain/Fulltext/2022/12000/A_consideration_of_differences_in_pain_scale
817   s_used.1.aspx

818   41. Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP. Validity of four pain intensity rating scales. *Pain*.
819   2011;152(10):2399-2404. doi:10.1016/j.pain.2011.07.005

820   42. Euasobhon P, Atisook R, Bumrungchatudom K, Zinboonyahgoon N, Saisavoey N, Jensen MP.
821   Reliability and responsivity of pain intensity scales in individuals with chronic pain. *PAIN*.
822   2022;163(12).
823   https://journals.lww.com/pain/Fulltext/2022/12000/Reliability_and_responsivity_of_pain_intensity
824   .6.aspx

825   43. Edlund MJ, Austen MA, Sullivan MD, et al. Patterns of opioid use for chronic noncancer pain in the
826   Veterans Health Administration from 2009 to 2011. *Pain*. 2014;155(11):2337-2343.
827   doi:10.1016/j.pain.2014.08.033

828   44. Department of Veterans Affairs. Pain Management - VHA Directive 2009-053. Published online
829   October 28, 2009. https://www.va.gov/painmanagement/docs/vha09paindirective.pdf

830   45. Hunter-Zinck H, Shi Y, Li M, et al. Genotyping Array Design and Data Quality Control in the Million
831   Veteran Program. *The American Journal of Human Genetics*. 2020;106(4):535-548.
832   doi:10.1016/j.ajhg.2020.03.004

833   46. Delaneau O, Zagury JF, Robinson MR, Marchini JL, Dermitzakis ET. Accurate, scalable and integrative
834   haplotype estimation. *Nature Communications*. 2019;10(1):5436. doi:10.1038/s41467-019-13225-y

835   47. Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and methods.
836   *Nature Genetics*. 2016;48(10):1284-1287. doi:10.1038/ng.3656

837   48. Auton A, Abecasis GR, Altshuler DM, et al. A global reference for human genetic variation. *Nature*.
838   2015;526(7571):68-74. doi:10.1038/nature15393

839   49. Fang H, Hui Q, Lynch J, et al. Harmonizing Genetic Ancestry and Self-identified Race/Ethnicity in
840   Genome-wide Association Studies. *The American Journal of Human Genetics*. 2019;105(4):763-772.
841   doi:10.1016/j.ajhg.2019.08.012

842   50. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the
843   challenge of larger and richer datasets. *GigaScience*. 2015;4(1):s13742-015-0047-0048.
844   doi:10.1186/s13742-015-0047-8

845   51. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association
846   scans. *Bioinformatics*. 2010;26(17):2190-2191. doi:10.1093/bioinformatics/btq340

847   52. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of
848   genetic associations with FUMA. *Nature Communications*. 2017;8(1):1826. doi:10.1038/s41467-017-
849   01261-5

850   53. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am
851   J Hum Genet*. 2011;88(1):76-82. doi:10.1016/j.ajhg.2010.11.011

852   54. Benner C, Spencer CCA, Havulinna AS, Salomaa V, Ripatti S, Pirinen M. FINEMAP: efficient variable
853   selection using summary data from genome-wide association studies. *Bioinformatics*.
854   2016;32(10):1493-1501. doi:10.1093/bioinformatics/btw018

855   55. Benner C, Havulinna AS, Järvelin MR, Salomaa V, Ripatti S, Pirinen M. Prospects of Fine-Mapping
856   Trait-Associated Genomic Regions by Using Summary Statistics from Genome-wide Association
857   Studies. *The American Journal of Human Genetics*. 2017;101(4):539-551.
858   doi:10.1016/j.ajhg.2017.08.012

859   56. Bulik-Sullivan BK, Loh PR, Finucane HK, et al. LD Score regression distinguishes confounding from
860   polygenicity in genome-wide association studies. *Nature Genetics*. 2015;47(3):291-295.
861   doi:10.1038/ng.3211

862   57. Altshuler DM, Gibbs RA, Peltonen L, et al. Integrating common and rare genetic variation in diverse
863   human populations. *Nature*. 2010;467(7311):52-58. doi:10.1038/nature09298

864   58. Luo Y, Li X, Wang X, et al. Estimating heritability and its enrichment in tissue-specific gene sets in
865   admixed populations. *Human Molecular Genetics*. 2021;30(16):1521-1534.
866   doi:10.1093/hmg/ddab130

867   59. Finucane HK, Bulik-Sullivan B, Gusev A, et al. Partitioning heritability by functional annotation using
868   genome-wide association summary statistics. *Nature Genetics*. 2015;47(11):1228-1235.
869   doi:10.1038/ng.3404

870   60. Finucane HK, Reshef YA, Anttila V, et al. Heritability enrichment of specifically expressed genes
871   identifies disease-relevant tissues and cell types. *Nature Genetics*. 2018;50(4):621-629.
872   doi:10.1038/s41588-018-0081-4

873   61. The GTEx consortium, Aguet François, Anand Shankara, et al. The GTEx Consortium atlas of genetic
874   regulatory effects across human tissues. *Science*. 2020;369(6509):1318-1330.
875   doi:10.1126/science.aaz1776

876   62. Bernstein BE, Stamatoyannopoulos JA, Costello JF, et al. The NIH Roadmap Epigenomics Mapping
877   Consortium. *Nat Biotechnol*. 2010;28(10):1045-1048. doi:10.1038/nbt1010-1045

878   63. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: Generalized Gene-Set Analysis of GWAS
879   Data. *PLOS Computational Biology*. 2015;11(4):e1004219. doi:10.1371/journal.pcbi.1004219

880   64. Sey NYA, Hu B, Mah W, et al. A computational tool (H-MAGMA) for improved prediction of brain-
881   disorder risk genes by incorporating brain chromatin interaction profiles. *Nature Neuroscience*.
882   2020;23(4):583-593. doi:10.1038/s41593-020-0603-0

883   65. Rajarajan P, Borrman T, Liao W, et al. Neuron-specific signatures in the chromosomal connectome
884   associated with schizophrenia risk. *Science*. 2018;362(6420). doi:10.1126/science.aat4311

885   66. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures
886   Database (MSigDB) hallmark gene set collection. *Cell Syst*. 2015;1(6):417-425.
887   doi:10.1016/j.cels.2015.12.004

888   67. Watanabe K, Umićević Mirkov M, de Leeuw CA, van den Heuvel MP, Posthuma D. Genetic mapping
889   of cell type specificity for complex traits. *Nature Communications*. 2019;10(1):3222.
890   doi:10.1038/s41467-019-11181-1

891   68. Wingo AP, Liu Y, Gerasimov ES, et al. Integrating human brain proteomes with genome-wide
892   association data implicates new proteins in Alzheimer's disease pathogenesis. *Nature Genetics*.
893   2021;53(2):143-146. doi:10.1038/s41588-020-00773-z

894   69. Fromer M, Roussos P, Sieberts SK, et al. Gene expression elucidates functional impact of polygenic
895   risk for schizophrenia. *Nature Neuroscience*. 2016;19(11):1442-1453. doi:10.1038/nn.4399

896   70. Wingo TS, Liu Y, Gerasimov ES, et al. Shared mechanisms across the major psychiatric and
897   neurodegenerative diseases. *Nature Communications*. 2022;13(1):4314. doi:10.1038/s41467-022-
898   31873-5

899   71. Gusev A, Ko A, Shi H, et al. Integrative approaches for large-scale transcriptome-wide association
900   studies. *Nature Genetics*. 2016;48(3):245-252. doi:10.1038/ng.3506

901   72. Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian Test for Colocalisation between Pairs of
902   Genetic Association Studies Using Summary Statistics. *PLOS Genetics*. 2014;10(5):e1004383.
903   doi:10.1371/journal.pgen.1004383

904   73. Zhu Z, Zhang F, Hu H, et al. Integration of summary data from GWAS and eQTL studies predicts
905   complex trait gene targets. *Nature Genetics*. 2016;48(5):481-487. doi:10.1038/ng.3538

906   74. Qi T, Wu Y, Fang H, et al. Genetic control of RNA splicing and its distinct role in complex trait
907   variation. *Nature Genetics*. 2022;54(9):1355-1363. doi:10.1038/s41588-022-01154-4

908   75. Ray P, Torck A, Quigley L, et al. Comparative transcriptome profiling of the human and mouse dorsal
909   root ganglia: an RNA-seq–based resource for pain and sensory neuroscience research. *PAIN*.
910   2018;159(7).
911   https://journals.lww.com/pain/Fulltext/2018/07000/Comparative_transcriptome_profiling_of_the_
912   human.16.aspx

913   76. Finan C, Gaulton A, Kruger FA, et al. The druggable genome and support for target identification and
914   validation in drug development. *Sci Transl Med*. 2017;9(383). doi:10.1126/scitranslmed.aag1166

915   77. Gaulton A, Bellis LJ, Bento AP, et al. ChEMBL: a large-scale bioactivity database for drug discovery.
916   *Nucleic Acids Res*. 2012;40(Database issue):D1100-1107. doi:10.1093/nar/gkr777

917   78. Cotto KC, Wagner AH, Feng YY, et al. DGIdb 3.0: a redesign and expansion of the drug–gene
918   interaction database. *Nucleic Acids Research*. 2018;46(D1):D1068-D1073. doi:10.1093/nar/gkx1143

919   79. Cuéllar-Partida G, Lundberg M, Kho PF, et al. Complex-Traits Genetics Virtual Lab: A community-
920   driven web platform for post-GWAS analyses. *bioRxiv*. Published online January 1, 2019:518027.
921   doi:10.1101/518027

922   80. Brown BC, Ye CJ, Price AL, Zaitlen N. Transethnic Genetic-Correlation Estimates from Summary
923   Statistics. *The American Journal of Human Genetics*. 2016;99(1):76-88.
924   doi:10.1016/j.ajhg.2016.05.001

925   81. Kember RL, Hartwell EE, Xu H, et al. Phenome-wide Association Analysis of Substance Use Disorders
926   in a Deeply Phenotyped Sample. *Biological Psychiatry*. doi:10.1016/j.biopsych.2022.08.010

927   82. Pierucci-Lagha A, Gelernter J, Feinn R, et al. Diagnostic reliability of the Semi-structured Assessment
928   for Drug Dependence and Alcoholism (SSADDA). *Drug Alcohol Depend*. 2005;80(3):303-312.
929   doi:10.1016/j.drugalcdep.2005.04.005

930   83. Pierucci-Lagha A, Gelernter J, Chan G, et al. Reliability of DSM-IV diagnostic criteria using the semi-
931   structured assessment for drug dependence and alcoholism (SSADDA). *Drug Alcohol Depend*.
932   2007;91(1):85-90. doi:10.1016/j.drugalcdep.2007.04.014

933   84. Fuchsberger C, Abecasis GR, Hinds DA. minimac2: faster genotype imputation. *Bioinformatics*.
934   2015;31(5):782-784. doi:10.1093/bioinformatics/btu704

935   85. Verma A, Damrauer SM, Naseer N, et al. The Penn Medicine BioBank: Towards a Genomics-Enabled
936   Learning Healthcare System to Accelerate Precision Medicine in a Diverse Population. *Journal of*
937   *Personalized Medicine*. 2022;12(12). doi:10.3390/jpm12121974

938   86. Ge T, Chen CY, Ni Y, Feng YCA, Smoller JW. Polygenic prediction via Bayesian regression and
939   continuous shrinkage priors. *Nature Communications*. 2019;10(1):1776. doi:10.1038/s41467-019-
940   09718-5

941   87. Denny JC, Ritchie MD, Basford MA, et al. PheWAS: demonstrating the feasibility of a phenome-wide
942   scan to discover gene–disease associations. *Bioinformatics*. 2010;26(9):1205-1210.
943   doi:10.1093/bioinformatics/btq126

944   88. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference
945   across the human phenome. *Elife*. 2018;7. doi:10.7554/eLife.34408

946   89. Tsepilov YA, Freidin MB, Shadrina AS, et al. Analysis of genetically independent phenotypes
947   identifies shared genetic factors associated with chronic musculoskeletal pain conditions. *Commun*
948   *Biol*. 2020;3(1):329. doi:10.1038/s42003-020-1051-9

949   90. Valdes AM, Abhishek A, Muir K, Zhang W, Maciewicz RA, Doherty M. Association of Beta-Blocker
950   Use With Less Prevalent Joint Pain and Lower Opioid Requirement in People With Osteoarthritis.
951   *Arthritis Care Res (Hoboken)*. 2017;69(7):1076-1081. doi:10.1002/acr.23091

952   91. Nakafero G, Grainge MJ, Valdes AM, et al. β-blocker prescription is associated with lower
953   cumulative risk of knee osteoarthritis and knee pain consultations in primary care: a propensity
954   score-matched cohort study. *Rheumatology (Oxford)*. 2021;60(12):5686-5696.
955   doi:10.1093/rheumatology/keab234

956   92. Jackson JL, Cogbill E, Santana-Davila R, et al. A Comparative Effectiveness Meta-Analysis of Drugs for
957   the Prophylaxis of Migraine Headache. *PLOS ONE*. 2015;10(7):e0130733.
958   doi:10.1371/journal.pone.0130733

959   93. Diatchenko L, Parisien M, Jahangiri Esfahani S, Mogil JS. Omics approaches to discover
960   pathophysiological pathways contributing to human pain. *PAIN*. 2022;163(S1).
961   https://journals.lww.com/pain/Fulltext/2022/11001/Omics_approaches_to_discover_pathophysiol
962   ogical.7.aspx

963   94. Mayr A, Jahn P, Stankewitz A, et al. Patients with chronic pain exhibit individually unique cortical
964   signatures of pain encoding. *Human Brain Mapping*. 2022;43(5):1676-1693. doi:10.1002/hbm.25750

965   95. Lee JJ, Lee S, Lee DH, Woo CW. Functional brain reconfiguration during sustained pain. Ploner M,
966   Behrens TE, Ploner M, Spisak T, eds. *eLife*. 2022;11:e74463. doi:10.7554/eLife.74463

967   96. Baliki MN, Chialvo DR, Geha PY, et al. Chronic Pain and the Emotional Brain: Specific Brain Activity
968   Associated with Spontaneous Fluctuations of Intensity of Chronic Back Pain. *J Neurosci*.
969   2006;26(47):12165. doi:10.1523/JNEUROSCI.3576-06.2006

970   97. Bortsov AV, Parisien M, Khoury S, et al. Brain-specific genes contribute to chronic but not to acute
971   back pain. *PAIN Reports*. 2022;7(5).
972   https://journals.lww.com/painrpts/Fulltext/2022/10000/Brain_specific_genes_contribute_to_chron
973   ic_but_not.6.aspx

974   98. Khoury S, Parisien M, Thompson SJ, et al. Genome-wide analysis identifies impaired axonogenesis in
975   chronic overlapping pain conditions. *Brain*. 2022;145(3):1111-1123. doi:10.1093/brain/awab359

976   99. Enna SJ, McCarson KE. The role of GABA in the mediation and perception of pain. *Adv Pharmacol*.
977   2006;54:1-27. doi:10.1016/s1054-3589(06)54001-3

978   100.   Goudet C, Magnaghi V, Landry M, Nagy F, Gereau RW 4th, Pin JP. Metabotropic receptors for
979   glutamate and GABA in pain. *Brain Res Rev*. 2009;60(1):43-56.
980   doi:10.1016/j.brainresrev.2008.12.007

981   101.   Dong W, Jin SC, Allocco A, et al. Exome Sequencing Implicates Impaired GABA Signaling and
982   Neuronal Ion Transport in Trigeminal Neuralgia. *iScience*. 2020;23(10):101552.
983   doi:10.1016/j.isci.2020.101552

984   102.   Xie L, Wu H, Chen Q, et al. Divergent modulation of pain and anxiety by GABAergic neurons in
985   the ventrolateral periaqueductal gray and dorsal raphe. *Neuropsychopharmacology*. Published
986   online December 16, 2022. doi:10.1038/s41386-022-01520-0

987   103.   Bridge H, Stagg CJ, Near J, Lau C ieong, Zisner A, Cader MZ. Altered neurochemical coupling in
988   the occipital cortex in migraine with visual aura. *Cephalalgia*. 2015;35(11):1025-1030.
989   doi:10.1177/0333102414566860

990   104.   Foerster BR, Petrou M, Edden RAE, et al. Reduced insular γ-aminobutyric acid in fibromyalgia.
991   *Arthritis Rheum*. 2012;64(2):579-583. doi:10.1002/art.33339

105.   Wu X, Yuan J, Yang Y, et al. Elevated GABA level in the precuneus and its association with pain intensity in patients with postherpetic neuralgia: An initial proton magnetic resonance spectroscopy study. *Eur J Radiol*. 2022;157:110568. doi:10.1016/j.ejrad.2022.110568

106.   Naik AK, Pathirathna S, Jevtovic-Todorovic V. GABAA receptor modulation in dorsal root ganglia in vivo affects chronic pain after nerve injury. *Neuroscience*. 2008;154(4):1539-1553. doi:10.1016/j.neuroscience.2008.04.061

107.   Yang J, Hall JE, Jose PA, Chen K, Zeng C. Comprehensive insights in GRK4 and hypertension: From mechanisms to potential therapeutics. *Pharmacology & Therapeutics*. 2022;239:108194. doi:10.1016/j.pharmthera.2022.108194

108.   Olsen RB, Bruehl S, Nielsen CS, Rosseland LA, Eggen AE, Stubhaug A. Hypertension prevalence and diminished blood pressure–related hypoalgesia in individuals reporting chronic pain in a general population: The Tromsø Study. *PAIN*. 2013;154(2). https://journals.lww.com/pain/Fulltext/2013/02000/Hypertension_prevalence_and_diminished_bl ood.15.aspx

109.   Li CY, Lin WC, Lu CY, Chung YS, Cheng YC. Prevalence of pain in community-dwelling older adults with hypertension in the United States. *Scientific Reports*. 2022;12(1):8387. doi:10.1038/s41598-022-12331-0

110.   Geisheker MR, Heymann G, Wang T, et al. Hotspots of missense mutation identify neurodevelopmental disorder genes and functional domains. *Nature Neuroscience*. 2017;20(8):1043-1051. doi:10.1038/nn.4589

111.   Ismail V, Zachariassen LG, Godwin A, et al. Identification and functional evaluation of GRIA1 missense and truncation variants in individuals with ID: An emerging neurodevelopmental syndrome. *The American Journal of Human Genetics*. 2022;109(7):1217-1241. doi:10.1016/j.ajhg.2022.05.009
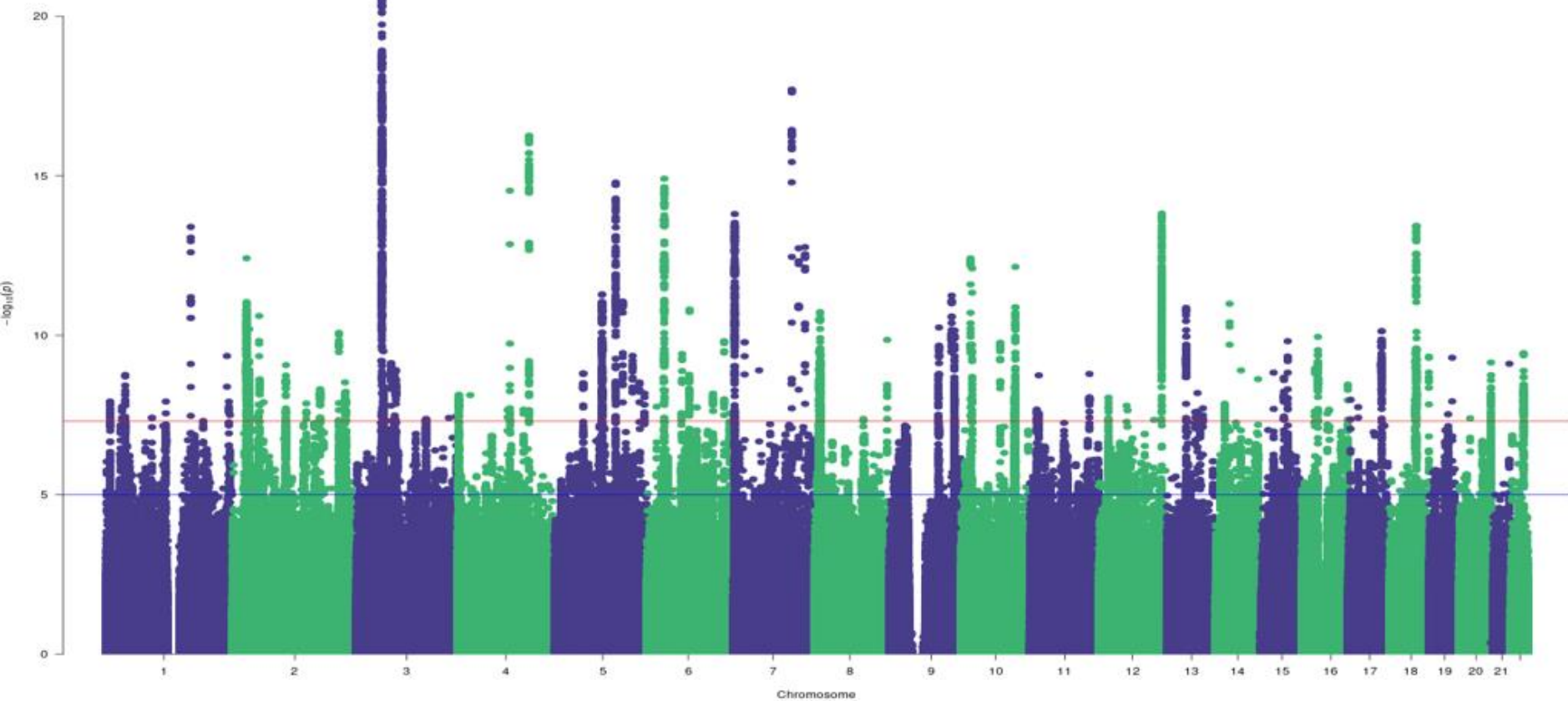
112.   Aranke M, Pham CT, Yilmaz M, et al. Topical Sevoflurane: A Novel Treatment for Chronic Pain Caused by Venous Stasis Ulcers. *Anesth Pain Med*. 2021;11(1):e112832. doi:10.5812/aapm.112832
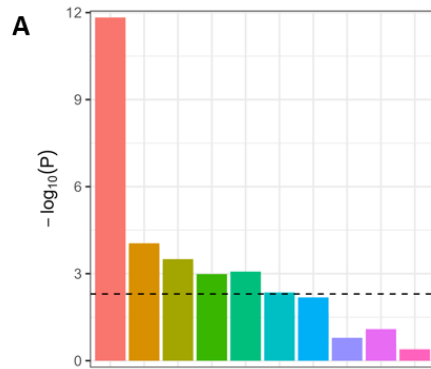
113.   Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *The Lancet Neurology*. 2015;14(2):162-173. doi:10.1016/S1474-4422(14)70251-0

114.   Khan JS, Hah JM, Mackey SC. Effects of smoking on patients with chronic pain: a propensity-weighted analysis on the Collaborative Health Outcomes Information Registry. *PAIN*. 2019;160(10). https://journals.lww.com/pain/Fulltext/2019/10000/Effects_of_smoking_on_patients_with_chronic _pain_.22.aspx

115.   Nicholas M, Vlaeyen JWS, Rief W, et al. The IASP classification of chronic pain for ICD-11: chronic primary pain. *Pain*. 2019;160(1):28-37. doi:10.1097/j.pain.0000000000001390

116.   Goulet JL, Brandt C, Crystal S, et al. Agreement between electronic medical record-based and self-administered pain numeric rating scale: clinical and research implications. *Med Care*. 2013;51(3):245-250. doi:10.1097/MLR.0b013e318277f1ad

1030

A

B