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Genetic polymorphism of pleiotrophin is associated with pain experience in Japanese adults

Case-control study

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Abstract

Genetic factors play a role in individual differences in pain experience. Here, we performed a genome-wide association study (GWAS) to identify novel loci regulating pain processing. We conducted a 2-stage GWAS and the candidate single-nucleotide polymorphisms (SNPs) association study on pain experience using an exploratory cohort of patients with cancer pain. The confirmatory cohort comprised of participants from the general population with and without habitual use of analgesic medication. In the exploratory cohort, we evaluated pain intensity using a numerical rating scale, recorded daily opioid dosages, and calculated pain reduction rate. In the confirmatory cohort, pain experience was defined as habitual nonsteroidal anti-inflammatory drug usage. Using linear regression models, we identified candidate SNP in the exploratory samples, and tested the association between phenotype and experienced pain in the confirmatory samples. We found 1 novel SNP (rs11764598)—located on the gene encoding for pleiotrophin on chromosome 7—that passed the genome-wide suggestive significance at 20% false discovery rate (FDR) correction in the exploratory samples of patients with cancer pain ($P = 1.31 \times 10^{-7}$, FDR = 0.101). We confirmed its significant association with daily analgesic usage in the confirmatory cohort ($P = .028$), although the minor allele affected pain experience in an opposite manner. We identified a novel genetic variant associated with pain experience. Further studies are required to validate the role of pleiotrophin in pain processing.

Abbreviations: FDR = false discovery rate, GWAS = Genome-wide association study, K-W test = Kruskal–Wallis test, M-H test = Mann–Whitney test, NRS = an 11-point numerical rating scale, SNPs = single-nucleotide polymorphisms.

Keywords: cancer pain, genetic polymorphism, nonsteroidal anti-inflammatory drug usage, pleiotrophin, PTN gene.

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1. Introduction

Studies have revealed that lower back, neck, other musculoskeletal, and osteoarthritis joint pain comprise a large part of the global health burden,[1] and pain is one of the most frequent causes for patients to seek medical care.[2] Chronic pain has thus been identified as a critical public health issue and a global health research priority.[3] The experience of pain is characterized by inter-individual variability. One of the examples is cancer-related pain, where the World Health Organisation cancer pain management guidelines have recommended that the individual experience and differential expression of pain be acknowledged and treated with individual doses of analgesics.[4]

Such individual differences in pain experiences are partly influenced by environmental factors such as diet and lifestyle; however, genotype also plays a role.[5] Human twin studies and other genetic studies have indicated that the heritability of chronic pain ranges from 30 to 70%.[6] Approximately 37%, 52 to 68%, and 35 to 58% of cases of neuropathic pain, lower back pain and neck pain, respectively, might be heritable.[7–9] Analyzing the influence of genetic factors through genetic investigation makes it possible to identify new molecules associated with pain, disentangle new mechanisms, and ideally create tailor-made treatments. For example, the mechanism behind chemotherapy-induced peripheral neuropathy was reported to be associated with several single-nucleotide polymorphisms (SNPs), suggesting specific networks for each chemotherapy agent.[10,11] With a similar approach, using genetic polymorphisms can lead to a better understanding of the differences in pain phenotypes of clinical patients and promote novel drug developments.[12]

Among genetic polymorphisms, SNPs are most often analyzed in pain medicine. Some SNPs associated with pain sensitivity and opioid sensitivity have been reported.[13] Here, we performed a genome-wide association study (GWAS) to identify novel loci regulating pain processing in 2 independent cohorts, with the purpose of increasing our fundamental understanding of pathological pain mechanisms. We first conducted the exploratory GWAS in patients with cancer pain and subsequently validated the relevant SNPs in the general population.

2. Materials and Methods

2.1. Exploratory study

2.1.1. Participants. The study protocol was approved by the institutional review board at each hospital belonging to Japanese TR-Cancer Pain Research Consortium (representative, the Ethics Committee, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo), and written informed consent was obtained from all participants. The inclusion criteria were as follows: (a) diagnosis of cancer pain (irrespective of the organ and pathology of malignant lesions), (b) cancer pain intensity on an 11-point numerical rating scale (NRS) (0 = no pain, 10 = worst possible pain), (c) pain duration > 1 week (recoded at inclusion), and (d) age > 20 years. Both opioid-naïve patients and patients with opioid use could participate in this study. We evaluated their pain intensity (NRS) twice before and after first prescribing or increasing opioid analgesics. The attending physician—an expert in cancer pain management—adjusted opioid dosages without a protocol for each individual patient at their discretion. Decrease in pain intensity from the first survey (at enrollment) to the second survey was expressed in percentage terms. We recorded the total and increased daily opioid dosages (converted into daily intravenous fentanyl equivalents, which was normalized to the patients' body weight) between the enrollment and the second survey. At the second survey—a day after adjusting opioid dosages—we evaluated pain intensity, recorded the increased opioid dosage, and calculated the decrease in pain intensity in percentage terms. The exclusion criteria were as follows: (a) patients with slight or more severe cognitive dysfunction, (b) patients with clinically relevant brain metastasis, and (3) suspicion of an origin of pain other than from cancer. We enrolled 90 patients [age, 58.4 ± 13.4 years (mean ± SD); female, 50; pain duration, 11.2 ± 18.8 months] from September 2010 to March 2012.

2.1.2. Genotyping. Venous blood samples were collected, and genomic DNA was isolated from peripheral blood lymphocytes using a standard salting-out procedure. The whole-genomic DNA was amplified, subsequently fragmented, and the denatured DNA was hybridized to a prepared Omni1-Quad BeadChip (Illumina, San Diego, CA), containing 1140,419 markers, for genotyping of all subjects. Normalised bead-intensity data obtained for each sample were loaded into GenomeStudio software (Genotyping module ver. 1.8.4; Illumina), which converted fluorescence intensities into SNP genotypes. One subject did not meet the criteria for quality control of genotyping. We excluded SNPs with a call frequency of <95%, with a deviation from Hardy-Weinberg equilibrium at type I error rate of less than 10^{-3} and with a minor allele frequency less than 10^{-3}, resulting in 771,433 SNPs.

2.1.3. Statistical analysis of individual genotype data. Statistical calculations for individual genotyping data were performed using plink version 1.07[14] (http://pngu.mgh.harvard.edu/purcell/plink/, Purcell et al 2007) R package version 2.14.1 (http://www.r-project.org; R Development Core Team 2013), EIGENSOFT package version 3.1,[15] and Haploview version 4.2.[16] Individual SNP associations with pain intensity were estimated using a linear regression model for additive model (each copy of the minor allele has an equivalent additional additive value, i.e., 0, 1, 2). In the linear regression model, we set pain intensity as the dependent variable and age, sex, body weight, and respective SNPs as covariates ($y = \beta\ 0 + \beta\ 1 \times \text{Age} + \beta\ 2 \times \text{Sex} + \beta\ 3 \times \text{Body weight} + \beta\ 4 \times \text{SNPs} + \varepsilon$).

Associations were considered significant at corrected $P < .05$. For the correction of multiple testing in the GWAS, false discovery rate (FDR) was calculated using the Benjamini-Hochberg step-up method.[17] A threshold of 20% FDR, corresponding to crude $P = 2.59 \times 10^{-7}$, was used to suggest significant associations because of the exploratory nature of this analysis.[18]

About the identified candidate SNPs, we analyzed their association with pain intensity, daily opioid dosages, and opioid analgesic responsiveness (i.e., pain decrease corresponding to increased opioid analgesics) using nonparametric statistics (i.e., Mann–Whitney (M-H) test for 2 groups; and Kruskal–Wallis (K-W) test and post hoc Bonferroni test for 3 groups). Results were considered significant at $P < .05$.

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2.2. Confirmatory stage

We analyzed the association of the candidate SNPs with daily analgesic usage using logistic regression analysis, 14,078 samples from the Japan Multi-Institutional Collaborative Cohort (J-MICC) study were included. The appropriate institutional review board at the hospital approved the study protocol, and all participants provided written informed consent. The detailed protocols have been reported in the previous report.[19] Briefly, the J-MICC study recruited participants from the general population (n = 92,647) persons aged 35 to 69 years from all over Japan. They answered a questionnaire about their lifestyles, medical history, and medication status; and donated a blood sample. Of 14,539 volunteers who were randomly selected to be genotyped, 14,078 subjects were analyzed in this study after quality control filters (the dataset used in the present study was fixed on August 19, 2020). Based on the habitual nonsteroidal antiinflammatory drug usage from the medication status, the participants were categorized into 2 groups: One included participant that had pain with any etiology, where the pain interfered with their daily activities; the other included participants without pain. Associations between habitual analgesic medication usage and the 3 genotypes from the exploratory SNP analysis were respectively analyzed using the chi-square test. The odds ratio for habitual analgesic usage was calculated after adjusting for sex and age. P values for all the relevant SNPs were corrected using the Bonferroni correction for multiple comparisons (if required).

The details of the statistical analysis (including both exploratory and confirmatory studies) were approved by all authors before analyses began.

3. Results

3.1. Exploratory study

Figure 1 shows the distribution of the -log10 P values of each SNP for all chromosomes (Manhattan plot) from the GWAS analysis. Of 771,433 SNPs analyzed in the additive model, 1 SNP (rs11764598), passed the genome-wide suggestive significance at 20% FDR correction ($P = 1.31 \times 10^{-7}$, which corresponded to FDR = 0.101). This SNP was located on a gene encoding pleiotrophin (*PTN*) on chromosome 7.

For rs11764598, 62 patients had major homozygosity [age, 59.8 ± 12.4 years; female, 36; body weight, 55.4 ± 10.3 kg], 21 patients had heterozygosity [age, 55.8 ± 15.4 years; female, 14; body weight, 55.7 ± 12.1 kg], and 5 patients had minor homozygosity [age, 50.8 ± 14.4 years; female, 3; body weight, 48.7 ± 1.5 kg]. Patients with major allele homozygosity had more severe pain (6.3 ± 1.7) than the other groups (hetero 4.6 ± 1.4, Bonferroni test: $P < .0001$; minor homo 3.0 ± 0.7, K-W test, Bonferroni test: $P < .0001$; Fig. 2a). Pain intensity of the patients who were heterozygous for this SNP was comparable to those with minor allele homozygosity (Bonferroni test: $P = .122$). Daily opioid requirements at the enrollment were comparable among the 3 groups (K-W test: $P = .370$; Fig. 2b). Although the increased opioid dosages were comparable (K-W test: $P = .318$), the pain reduction rate was less in patients with minor allele homozygosity than those with major homozygosity and heterozygosity (K-W test: $P = .028$; Bonferroni test: vs major, $P < .0001$ and vs hetero, $P = .004$; Figs. 2c and 2d).

3.2. Confirmatory study

For the rs11764598 SNP, 9155 subjects had major homozygosity [age, 54.8 ± 9.4 years; female, 5052; body weight, 59.5 ± 11.3 kg], 4365 subjects had heterozygosity [age, 54.7 ± 9.4 years; female, 2372; body weight, 59.9 ± 11.2], and 558 subjects had minor homozygosity [age, 55.1 ± 9.3 years; female, 318; body weight, 59.8 ± 11.1 kg]. Of the 14,078 subjects, 500 subjects used analgesics habitually. The rs11764598 genotypes were significantly associated with habitual analgesics usage (chi-square, $P = .028$; Table 1). Patients with minor allele homozygosity used analgesics more often than those with major allele homozygosity or heterozygosity. The odds ratio for habitual analgesic usage as a proxy for having pain (minor allele homozygosity vs major allele homozygosity) after adjusting for sex and age was 1.62 [95% CI: 1.10–2.38].

4. Discussion

Our present findings revealed that patients with major allele homozygosity for rs11764598 on the *PTN* gene had higher pain intensity compared to those with heterozygosity or minor allele homozygosity. We confirmed its association of the minor allele homozygosity for rs11764598 with daily analgesic usage in a confirmatory cohort. Our 2-stage strategy of exploration and replication thus suggests that pleiotrophin is a possible candidate for disentangling the mechanism(s) of pain processing although the minor allele affected pain experience in an opposite manner.

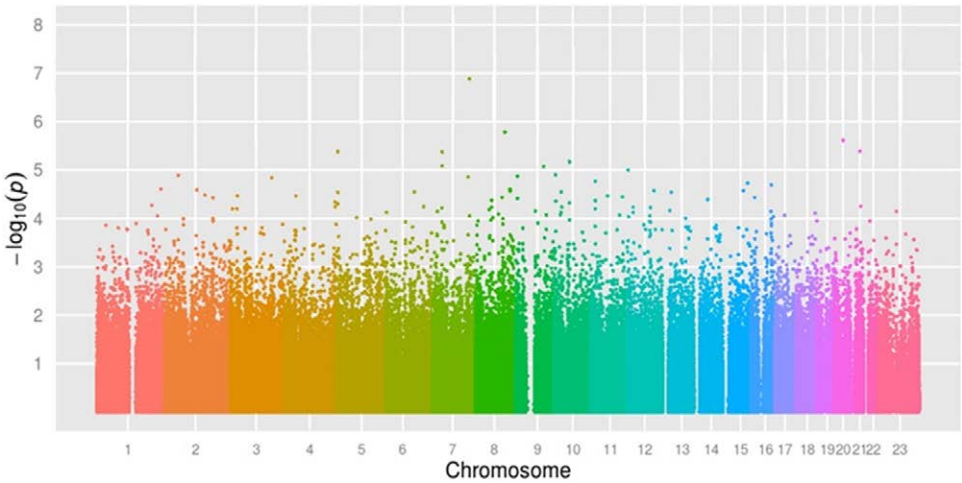

Figure 1. Manhattan plot of the genome-wide association study of cancer pain intensity from the exploratory samples (N = 89). The x-axis represents chromosomal positions, and the y-axis represents – log10 (P-values) calculated by a mixed linear model association analysis with cancer pain intensity. The horizontal red line indicates the genome-wide significance level ($P < 1.858 \times 10^{-7}$).

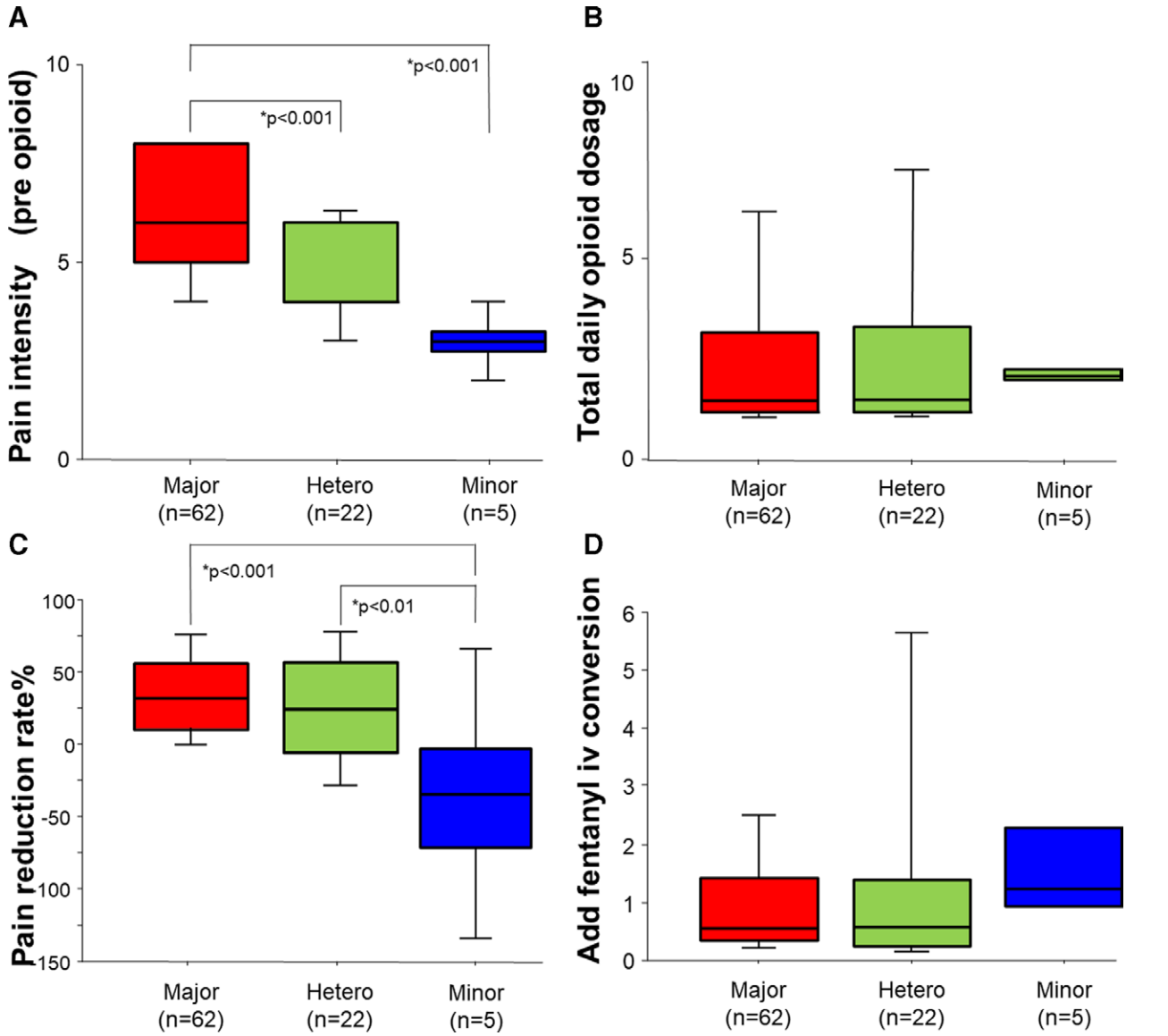

Figure 2. Associations between the polymorphism of rs11764598 and phenotypes. The boxes represent the 25th to 75th percentiles with the horizontal line showing the median value of the major allele homozygosity, heterozygosity, and minor allele homozygosity of the rs11764598 genetic polymorphism: (a) Cancer pain intensity at the baseline assessment; (b) daily total opioid dosages at the baseline assessment; (c) percentage decrease in cancer pain intensity after adjusting opioid dosages at the second survey; and (d) the increased opioid dosages at the second survey. Opioid dosages were converted into daily intravenous fentanyl equivalents, which was normalized to the patients' body weight.

Table 1

Genotypic model of the single nucleotide polymorphism (rs11764598) on the pleiotrophin gene associated with pain processing.

Cohort	Location (GRCh38)	Allele (major/minor)	Genotype	MAF		
Exploratory	7: 136602480	C/T	CC (62) CT (22)TT (5)	0.180		
Cohort			Genotype (Case/Control)	MAF (Case/Control)	*P* (χ^2)	Odds ratio [95% CI]
Confirmatory			CC (306/8849) CT (164/4201)TT (30/528)	0.224/0.194	0.0283 (7.1316)	1.62 [1.10–2.38]

The odds ratio represents minor allele homozygosity vs major allele homozygosity after adjusting for age and sex. The case represents subjects with habitual analgesic use as a proxy for having pain.
CI = confidence interval, MAF = minor allele frequency, SNP = single-nucleotide polymorphism.
*The chi-square test was performed for major, minor, and hetero groups between the case and control; significance was set at $P < 0.05$.

Pleiotrophin is one of the several neurotrophic factors that are the driving forces behind neuroplasticity. Pleiotrophin promotes neurite growth,[20] modulates synaptic transmission and is involved in long-term potentiation in learning and memory.[21,22] The present study raises an interesting question about the possibly bidirectional actions (i.e., promoting and inhibitory actions) of pleiotrophin on pain processing. In the exploratory cohort, the minor allele of the SNP decreased the risk for debilitating pain intensity. Conversely, in the confirmatory cohort, the subjects with the minor allele increased daily usage of nonopioid analgesics, indicating more severe pain prevalence.

From 1 view of pain processing, pleiotrophin would have a promoting effect. Genetic deletion of *PTN* could reduce nociceptive response at the level of the spinal dorsal horn but not the supra-spinal level.[23] Pleiotrophin is also reportedly involved in microglia-mediated neuroinflammation,[24–26] indicating reinforcement of pain processing because neuron-glia interaction in the spinal dorsal horn enhances nociceptive transmission. In addition to its neurotrophic activity, pleiotrophin could induce the expression of pro-inflammatory cytokines and trigger tissue inflammation.[27–30]

Conversely, pleiotrophin could have an inhibitory effect on pain processing. Pleiotrophin, which is highly expressed on ganglions in the peripheral nervous system and the dorsal root ganglia could alleviate allodynia and hyperalgesia in neuropathic pain.[31,32] Further, pleiotrophin can suppress the expression of cyclooxygenase-2 and tumor necrosis factor-alpha, both of which are involved in inflammation.[33]

Thus, previous studies have demonstrated that pleiotrophin acts on pain processing in a bidirectional manner, although the mechanisms for this have not been fully elucidated. Moreover, patients with the minor allele of the *PTN* gene showed attenuated opioid sensitivity. In *PTN* knockout mice, either morphine- or alpha 2-adrenergic receptor agonist-induced analgesia was significantly enhanced, suggesting a key role of pleiotrophin in the endogenous pain descending inhibitory pathways.[34] Therefore, our present findings are a step toward a better understanding of the pleiotrophin's role in modifying pain processing, including nociceptive transmission, endogenous descending pain inhibitory system, and inflammation.

To our knowledge, there are no previous studies where the SNP rs11764598 contributes to the functional alteration of pleiotrophin. Anecdotal evidence comes from previous reports suggesting that other genetic polymorphisms of the *PTN* gene are associated with bone mineral density[35] and body mass index.[36] Our present findings indicate that a relevant SNP of the *PTN* gene alters the functional property of pleiotrophin, but this should be confirmed in a future study, with larger cohorts of participants with homogeneous pain etiology.

In summary, we found 1 SNP in the *PTN* gene, which is involved in pain processing. In cancer pain patients, those with the major allele homozygosity had more severe pain than other patients. Opioid analgesia was less in patients with the minor allele homozygosity than other patients although the increased opioid dosages were comparable. In general population, the prevalence of nonsteroidal antiinflammatory drug users among those with the minor allele homozygosity were higher than those with the major allele homozygosity. Thus, our present study revealed that the minor allele of the *PTN* gene affects pain experience in an opposite manner. Further studies should focus on how pleiotrophin influences pain transmission, opioid sensitivity, and the descending pain inhibitory system and inflammation, in order to disentangle the more precise mechanisms involved.

Author contributions

Concept and design: MS, KW and KI
Data acquisition: All authors
Drafting of paper: KS, YS, DN, KI, TT, KW and MS
Critical revision of the manuscript: All authors
Data interpretation and statistical analysis: DN, TT
Supervision: MS and KU

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