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Genome-wide association study identifies candidate loci associated with chronic pain and postherpetic neuralgia

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Abstract

Background: Human twin studies and other studies have indicated that chronic pain has heritability that ranges from 30% to 70%. We aimed to identify potential genetic variants that contribute to the susceptibility to chronic pain and efficacy of administered drugs. We conducted genome-wide association studies (GWASs) using whole-genome genotyping arrays with more than 700,000 markers in 191 chronic pain patients and a subgroup of 89 patients with postherpetic neuralgia (PHN) in addition to 282 healthy control subjects in several genetic models, followed by additional gene-based and gene-set analyses of the same phenotypes. We also performed a GWAS for the efficacy of drugs for the treatment of pain.

Results: Although none of the single-nucleotide polymorphisms (SNPs) were found to be genome-wide significantly associated with chronic pain ($p \geq 1.858 \times 10^{-7}$), the GWAS of PHN patients revealed that the rs4773840 SNP within the *ABCC4* gene region was significantly associated with PHN in the trend model (nominal $p = 1.638 \times 10^{-7}$). In the additional gene-based analysis, one gene, *PRKCQ*, was significantly associated with chronic pain in the trend model (adjusted $p = 0.03722$). In the gene-set analysis, several gene sets were significantly associated with chronic pain and PHN. No SNPs were significantly associated with the efficacy of any of types of drugs in any of the genetic models.

Conclusions: These results suggest that the *PRKCQ* gene and rs4773840 SNP within the *ABCC4* gene region may be related to the susceptibility to chronic pain conditions and PHN, respectively.

Keywords

Genome-wide association study, single-nucleotide polymorphism, chronic pain, postherpetic neuralgia, gene-based/gene-set analysis

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Introduction

An estimated 15–50% of the population experiences pain at any given time.[1–3] Some pain is acute or subacute, but other forms of pain are chronic.[4] Chronic pain is a public health problem that affects the general population physically, psychologically, and socially.[5] Chronic pain is prevalent among the Japanese population, affecting 15.4–47% of individuals.[5,6] The median prevalence of chronic pain was reported to be 26% among the adult population worldwide, ranging from 7% to 55%.[5] Chronic pain has been reported to be associated with health status, work productivity,

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impairments in daily activities, healthcare resource utilization, and economic burdens in Japan.[6] According to a recent report, people with chronic pain, particularly cancer-related pain, have a slightly higher risk of death.[7]

Chronic pain conditions are complex traits with multiple etiologies. With regard to non-genetic and nonheritable factors, regression analyses have shown that chronic pain is associated with age, sex, unemployment, living status, exercise,[5] body mass index, fatigue, sleep, and mobility problems.[3] Human twin studies and other genetic studies have indicated that the heritability of chronic pain ranges from 30% to 70%.[8] Approximately 37%, 52–68%, and 35–58% of cases of neuropathic pain, low back pain, and neck pain, respectively, may be heritable.[9,10] Previous genetic studies of candidate genes that are related to pain mechanisms found that human genetic variations were associated with various pain-related phenotypes.[1,11,12] Pain-related genetic variations have also been identified for chronic pain conditions, such as the *ADRB2*,[13,14] *HTR2A*,[15] *SCN9A*,[16] *KCNS1*,[17] *CACNA2D3*,[18] *CACNG2*,[19] *COMT*,[20] IL4,[14] and IL10[21] genes. Candidate genes for chronic postsurgical pain (CPSP) were systematically reviewed by Hoofwijk et al.,[22] and candidate genes for neuropathic pain have been described in several previous reports.[23–26] Chronic pain-related single-nucleotide polymorphisms (SNPs) have also been explored based on recent advances in high-density SNP arrays that can screen hundreds of thousands or millions of genetic markers throughout the human genome. For example, Jones et al. (2016) found that a SNP that was colocalized to the *NGF* gene, which encodes nerve growth factor, was associated with dysmenorrhea in a genome-wide association study (GWAS) of a cohort of females.[27] Peters et al. identified a common genetic variant on chromosome 5p15.2 that was associated with joint-specific chronic widespread pain (CWP) in a large-scale GWAS meta-analysis.[28] Genome-wide association studies have also been applied to investigate neuropathic pain. Several candidate loci were reported to be associated with pain conditions, including diabetic neuropathic pain.[29–32]

In the present study, we conducted GWASs of patients with chronic pain to identify potential genetic variants that contribute to the susceptibility to pain conditions and efficacy of several types of drugs that are used to treat pain. We also performed a GWAS to explore genetic factors that are associated with neuropathic pain, specifically postherpetic neuralgia (PHN).

Methods

Subjects with chronic pain and healthy subjects

We enrolled 194 adult patients who suffered from chronic pain who visited JR Tokyo General Hospital (Tokyo, Japan), Juntendo University Hospital (Tokyo, Japan), or Nihon University Itabashi Hospital (Tokyo, Japan) for the treatment of chronic pain and were apparently Japanese. Most of the patients were treated with analgesics before recruitment or were scheduled to be treated with analgesics at the time of recruitment in the study. We excluded patients with severe coexisting complications. The detailed demographic and clinical data of the subjects are provided in Table 1.

We also enrolled 282 healthy adult volunteers as controls who were disease-free, did not experience chronic pain, and who lived in or near the Kanto area in Japan. The detailed demographic data of the control subjects and their statistics are detailed in previous reports.[33,34]

The study protocol was approved by the Institutional Review Board of JR Tokyo General Hospital (Tokyo, Japan), Institutional Review Board of Juntendo University Hospital (Tokyo, Japan), Institutional Review Board of Nihon University Itabashi Hospital (Tokyo, Japan), and Institutional Review Board of Tokyo Metropolitan Institute of Medical Science (Tokyo, Japan). Written informed consent was obtained from all of the patients.

Patient characteristics and clinical data

In the patient subjects, we obtained data on surgical history, treatment history, pain status (e.g., presence/absence of nerve block and allodynia), drug treatments, and disease status (e.g., postherpetic neuralgia [PHN], spinal canal stenosis, lower back pain [LBP], etc.; Table 1). Some of the patients were affected by multiple diseases.

Various types of drugs were administered to the patients for the treatment of pain. In the present study, these drugs were divided into several groups for the analysis, including opioids (e.g., morphine and codeine), antidepressants (e.g., fluvoxamine and amitriptyline), anticonvulsants (e.g., gabapentin and pregabalin), nonsteroidal antiinflammatory drugs (NSAIDs; e.g., loxoprofen and diclofenac), γ-aminobutyric acid (GABA) receptor agonists that can be used as anticonvulsants or anxiolytics (e.g., clonazepam and diazepam), ketamine, neurotropin, lidocaine, and other drugs (e.g., Chinese herbal medicines and mexiletine). The detailed data on drug administration are provided in Table 1. Some patients received only one type of drug, whereas others received several types of drugs. Some of the drugs were effective for a number of patients, but others were not. Such drug administration and efficacy were comprehensively recorded for the statistical analyses.

Table 1. Demographic and clinical data of patient subjects.

Demographic data	n	Minimum	Maximum	Mean	SD	Median						
Gender of all patients												
Male	89											
Female	100											
Age (years)	193	22	89	65.18	13.95	68.00						
Weight (kg)	182	34	98	57.32	12.21	57.00						
Status of patients	Absence	Presence	Opioids	Antidepressant	Anticonvulsant	NSAIDs[†]	GABA[§]	Ketamine	Neurotropin	Lidocaine	Others	
Nerve block	132	25										
Allodynia	75	30										
Administration of drugs			50	66	99	25	58	7	5	18	4	
Diagnosis (disease status)		*n*				Diagnosis (disease status)					*n*	
Postherpetic neuralgia (PHN)		92				Spinal canal stenosis					20	
Lower back pain (LBP)		13				Postoperative pain					12	
Hernia of intervertebral disk		8				Neck pain					8	
Others		46										

[†]Non-steroidal anti-inflammatory drugs.
[§]Gamma-aminobutyric acid receptor modulators.

Whole-genome genotyping and quality control

A total of 194 DNA samples from the patients were used for genotyping. Total genomic DNA was extracted from whole-blood samples using standard procedures. Whole-genome genotyping was performed using the Infinium assay II with an iScan system (Illumina, San Diego, CA, USA) according to the manufacturer's instructions, and two kinds of BeadChips were used for genotyping 153 and 41 patient samples, respectively: HumanOmni1-Quad v1.0 (total markers: 11,34,514) and HumanOmniExpress-12 v1.1 (total markers: 7,19,665). For genotyping 282 control samples, the HumanOmniExpressExome-8 v1.2 BeadChip (total markers: 9,64,193) was used. Other details for genotyping are described in the Supplementary Methods. The data for the whole-genome-genotyped samples were analyzed using GenomeStudio with the Genotyping module v3.3.7 (Illumina) to evaluate the quality of the results. In the data-cleaning process as detailed in the Supplementary Methods, three patient samples were excluded from further analyses, whereas no control samples were excluded based on this criterion. For the study of the effects of drugs in patients, 4,47,634 SNPs survived the entire filtration process and were used in the study. For the case-control study to compare genotypes between the patient and control subjects, more stringent criteria were used for filtration to remove spurious results, and 445,723 SNPs survived the entire filtration process and were used in the study. Furthermore, the TaqMan allelic discrimination assay (Life Technologies, Carlsbad, CA, USA) was performed to confirm the genotype data of the top 20 candidate SNPs if the data were suspected to be dubious.

Statistical analysis

A GWAS of patients with chronic pain was conducted to investigate associations between genetic variations and the susceptibility to chronic pain in all 191 patient subjects who passed the quality control criteria. A GWAS of a subgroup of 89 patients with PHN was also conducted because PHN was the most prevalent pain condition in our samples. A total of 282 control subjects were used in both of these analyses. Furthermore, another GWAS of only 191 patient subjects was also conducted to investigate the effects of drugs.

To explore associations between SNPs and disease status, Fisher's exact tests were conducted in both analyses using both all patients and patients with PHN to compare genotype data between the patient and control subjects. To explore SNPs that were associated with the effects of drugs in patients, patient subjects were divided into two groups based on the effectiveness of five major

kinds of drugs (i.e., opioids, antidepressants, anticonvulsants, NSAIDs, and GABA receptor agonists; Table 1), and Fisher's exact tests were conducted to compare genotype data between the two groups. Trend, dominant, and recessive genetic models were used for all of the analyses because of insufficient knowledge of genetic factors that are associated with chronic pain, PHN, and the effectiveness of drugs that are used for the treatment of chronic pain. The association study included both female and male subjects for autosomal markers, although male genotypes were excluded from the analysis of X chromosome markers. All of the statistical analyses were performed using gPLINK v. 2.050, PLINK v. 1.07 (http://zzz.bwh.harvard.edu/plink/index.shtml; accessed July 15, 2018),[35] and Haploview v. 4.2.[36]

For the correction of multiple testing in the GWAS, Bonferroni correction was used for the number of inferred Meff, defined in simpleM software,[37–39] which is a multiple-testing correction method for genetic association studies that uses correlated SNPs. In our preliminary calculation, by substituting missing genotypes with homozygotes of minor or major alleles and heterozygotes, Meff was estimated to be 256,506–269,170. Therefore, statistical significance for the GWAS was defined as a corrected $p < 0.05/269{,}170 = 1.858 \times 10^{-7}$ in the present study.

To further understand the genetic backgrounds and molecular mechanisms that underlie complex traits, such as chronic pain and PHN, gene-based and gene-set approaches were adopted with Multi-marker Analysis of GenoMic Annotation (MAGMA) v1.06,[40] which is also available on the Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA GWAS) v1.3.3 platform,[41] as detailed in the Supplementary Methods. In the gene-set analysis, gene sets were defined using the Molecular Signatures Database (MSigDB) v6.1,[42] and a total of 10,654 gene sets (curated gene sets: 4737, GO terms: 5917) from MsigDB were tested.

Results

Identification of genetic polymorphisms associated with chronic pain and postherpetic neuralgia by GWAS

We comprehensively explored genetic variations that were associated with chronic pain conditions in a total of 191 patients who visited hospitals for treatment, and 282 adult healthy subjects were recruited as controls.[33,34] In the GWAS of all patients, 4,45,723 SNPs that passed the quality control criteria were selected as candidate genetic polymorphisms in the trend, dominant, and recessive models. Among the highly ranked SNPs, genotype data for one SNP, rs6481467, was suspected to be dubious because of its cluster separation. After screening using the TaqMan allelic discrimination assay, the data were found to be erroneous for this SNP and thus were removed from the list of candidate SNPs. Table 2 shows the top 20 candidate SNPs in each genetic model after final quality control. However, none of the SNPs were genome-wide significantly associated with the phenotype ($p \geq 1.858 \times 10^{-7}$; Table 2, Figure 1 (a)). We then conducted another GWAS of the same SNPs by including only a subgroup of 89 patients with PHN. A significant association was found between the rs4773840 SNP that mapped to 13q32.1 and PHN in the trend model (nominal $p = 1.638 \times 10^{-7}$; Table 3, Figure 1(b)). The calculated $\log_{10}$ values (observed p value) for most of the analyzed SNPs were in accordance with or below the expected values based on the null hypothesis of a uniform distribution in the QQ plot (Supplementary Figures S1 and S2). The values for the rs4773840 SNP and other SNPs that ranked high in Table 3 were obviously above the expected values (Supplementary Figure S2). The gene that was located in this region of the rs4773840 SNP was *ABCC4*, which encodes adenosine triphosphate binding cassette subfamily C member 4. Most of the other SNPs in this gene region that ranked high in Table 3 were in relatively strong linkage disequilibrium (LD) with one another, and all of these SNPs were within the *ABCC4* gene region (Figure 2). As shown in Table 3, an increment of the minor C allele carriage in the rs4773840 SNP was associated with a greater risk of PHN.

Identification of genes and gene sets associated with chronic pain and postherpetic neuralgia by gene-based and gene-set analyses

Considering the fact that the effects of individual markers tend to be too weak to be detected by comprehensive analyses, such as GWASs, that target only single polymorphisms, we conducted gene-based and gene-set analyses, which are statistical methods that are used to analyze multiple genetic markers simultaneously to determine their joint effect. In both analyses, we explored genes and gene sets that were associated with chronic pain conditions and PHN in a total of 191 patients, including 89 PHN patients and 282 control subjects, similarly to our GWAS by running MAGMA software,[40] which was available in the FUMA GWAS platform.[41] Consequently, the analyses of all patients included 4,45,723 SNPs of selected candidate genes and gene sets in the trend, dominant, and recessive models. Supplementary Tables S1 and S2 show the top 20 candidate genes that were identified in each genetic model in the gene-set analysis. The best candidate gene in the trend model that resulted from an analysis of all

Table 2. Top 20 candidate SNPs selected from GWAS for all patients.

Model	Rank	CHR	SNP	Position	*P*	Related gene	Genotype (patients)			Genotype (controls)		
							A/A	A/B	B/B	A/A	A/B	B/B
Trend	1	8	rs10086452	3691292	0.00001026	*CSMD1*	2	48	141	18	107	156
Trend	2	16	rs12708686	25789460	0.00001532	*HS3ST4*	5	70	116	28	133	121
Trend	3	10	rs688391	6529658	0.00001721	*PRKCQ*	59	105	27	61	126	95
Trend	4	16	rs9989408	25786610	0.0000198	*HS3ST4*	8	76	107	34	140	108
Trend	5	4	rs4141270	106242441	0.00002805		44	95	52	33	126	122
Trend	6	4	rs10518617	133841275	0.00003039		29	84	78	15	107	159
Trend	7	20	rs4811012	48294701	0.00003177		3	58	130	22	116	144
Trend	8	15	rs6493688	29560167	0.00003323		40	89	62	25	124	133
Trend	9	12	rs10844159	32288782	0.00003414	*BICD1*	21	81	89	10	94	178
Trend	10	1	rs10803183	242444561	0.00003789		5	58	128	6	36	240
Trend	11	13	rs4773840	94568426	0.00004323	*ABCC4*	22	80	89	10	96	176
Trend	12	14	rs11621135	70729362	0.00004646		10	65	115	2	66	214
Trend	13	17	rs2958927	50314685	0.00004719		29	81	77	17	104	161
Trend	14	13	rs1678353	94547567	0.00004959	*ABCC4*	23	81	87	9	103	170
Trend	15	10	rs4749828	9062151	0.00004966		15	80	95	6	89	187
Trend	16	7	rs12700309	21850980	0.00005138	*DNAH11*	57	99	35	48	144	90
Trend	17	10	rs17784350	50512270	0.00005223	*CHAT*	7	61	123	25	127	130
Trend	18	2	rs2693818	6121959	0.0000536		31	80	79	59	166	57
Trend	19	11	rs6265	27636492	0.00005366	*BDNF-AS1,BDNF*	40	107	44	34	136	112
Trend	19	11	rs11030104	27641093	0.00005366	*BDNF-AS1,BDNF*	40	107	44	34	136	112
Dominant	1	2	rs2693818	6121959	0.0000009002		31	80	79	59	166	57
Dominant	2	2	rs6718476	6112647	0.0000009454		31	81	79	59	166	57
Dominant	3	10	rs688391	6529658	0.000001239	*PRKCQ*	59	105	27	61	126	95
Dominant	4	10	rs604663	6544132	0.000002684	*PRKCQ*	52	110	29	57	128	97
Dominant	5	1	rs10803183	242444561	0.000005297		5	58	128	6	36	240
Dominant	6	11	rs1488830	27593461	0.00003125	*BDNF-AS1*	53	107	31	54	134	94
Dominant	7	18	rs12964456	30023916	0.00003475	*NOL4*	17	56	118	20	143	118
Dominant	8	4	rs6531299	33872088	0.00003526		14	82	95	14	74	194
Dominant	9	20	rs6133220	551620	0.00003676		36	114	41	38	132	112
Dominant	10	2	rs941009	6058737	0.00003957		25	83	83	54	157	71
Dominant	11	1	rs6656194	164031638	0.00004554		33	98	60	29	111	142
Dominant	12	7	rs6461595	21724570	0.0000477	*DNAH11*	41	111	39	53	122	107
Dominant	13	8	rs2433150	6489560	0.00005107		5	40	146	13	104	165
Dominant	14	13	rs9532107	37187961	0.00005386	*TRPC4*	14	67	110	33	140	109
Dominant	15	2	rs10204095	57652544	0.0000553		5	37	148	10	101	166
Dominant	16	4	rs7670109	184691188	0.00005679		38	87	66	74	157	51
Dominant	17	6	rs13196989	184373	0.00005703		8	74	108	10	61	211
Dominant	18	3	rs7610425	150967983	0.00005804	*ANKUB1*	9	90	92	11	82	189
Dominant	19	2	rs12468070	6077432	0.00006067		25	84	82	56	155	71

(continued)

Table 2. Continued.

Model	Rank	CHR	SNP	Position	*P*	Related gene	Genotype (patients)			Genotype (controls)		
							A/A	A/B	B/B	A/A	A/B	B/B
Dominant	20	14	rs2167151	78933086	0.00006216	*NRXN3*	17	84	90	14	82	186
Recessive	1	1	rs4520412	15232554	0.0000008571	*KAZN*	25	110	56	92	115	75
Recessive	2	11	rs1519480	27632288	0.000002159	*BDNF-AS1*	0	61	130	25	101	156
Recessive	3	6	rs3777799	133631276	0.000003063	*EYA4*	22	54	111	4	93	185
Recessive	4	8	rs12545634	26929236	0.00001289		39	77	75	19	121	142
Recessive	5	2	rs10205827	75356361	0.00002183		10	102	79	52	122	107
Recessive	6	2	rs10208470	75356624	0.00002186		10	102	79	52	122	108
Recessive	7	7	rs12538837	97522404	0.00004215		27	111	53	86	128	68
Recessive	8	8	rs10086635	26955860	0.00004484		48	76	67	30	142	110
Recessive	9	4	rs6826653	19736139	0.00004904		15	55	121	2	84	196
Recessive	10	2	rs9309489	75355228	0.00004915		11	101	79	52	122	108
Recessive	11	10	rs2026432	6547609	0.00004948	*PRKCQ*	25	106	60	81	130	71
Recessive	12	13	rs9521844	110018508	0.00005096		0	61	130	19	94	169
Recessive	13	9	rs10959456	11002926	0.00005841		0	66	120	19	105	158
Recessive	14	8	rs9314506	3682052	0.00007367	*CSMD1*	25	102	64	80	131	71
Recessive	15	13	rs9555965	89459182	0.00007841		39	72	80	22	121	139
Recessive	15	13	rs9555966	89460007	0.00007841		39	72	80	22	121	139
Recessive	17	6	rs13203299	169184034	0.00008602		33	68	90	16	122	144
Recessive	18	11	rs12291063	27650677	0.00009339	*BDNF-AS1,BDNF*	0	53	138	18	92	172
Recessive	19	22	rs7290832	25658787	0.00009952		38	81	72	21	149	112
Recessive	20	9	rs871095	138095067	0.0001101	*NACC2*	41	93	57	24	145	113

Model, the genetic model in which candidate SNPs were selected by GWAS; CHR, chromosome number.
Related gene, the nearest gene from the SNP site; A/A, homozygote for the minor allele in each SNP.
A/B, heterozygote for the major allele in each SNP; B/B, homozygote for the major allele in each SNP.

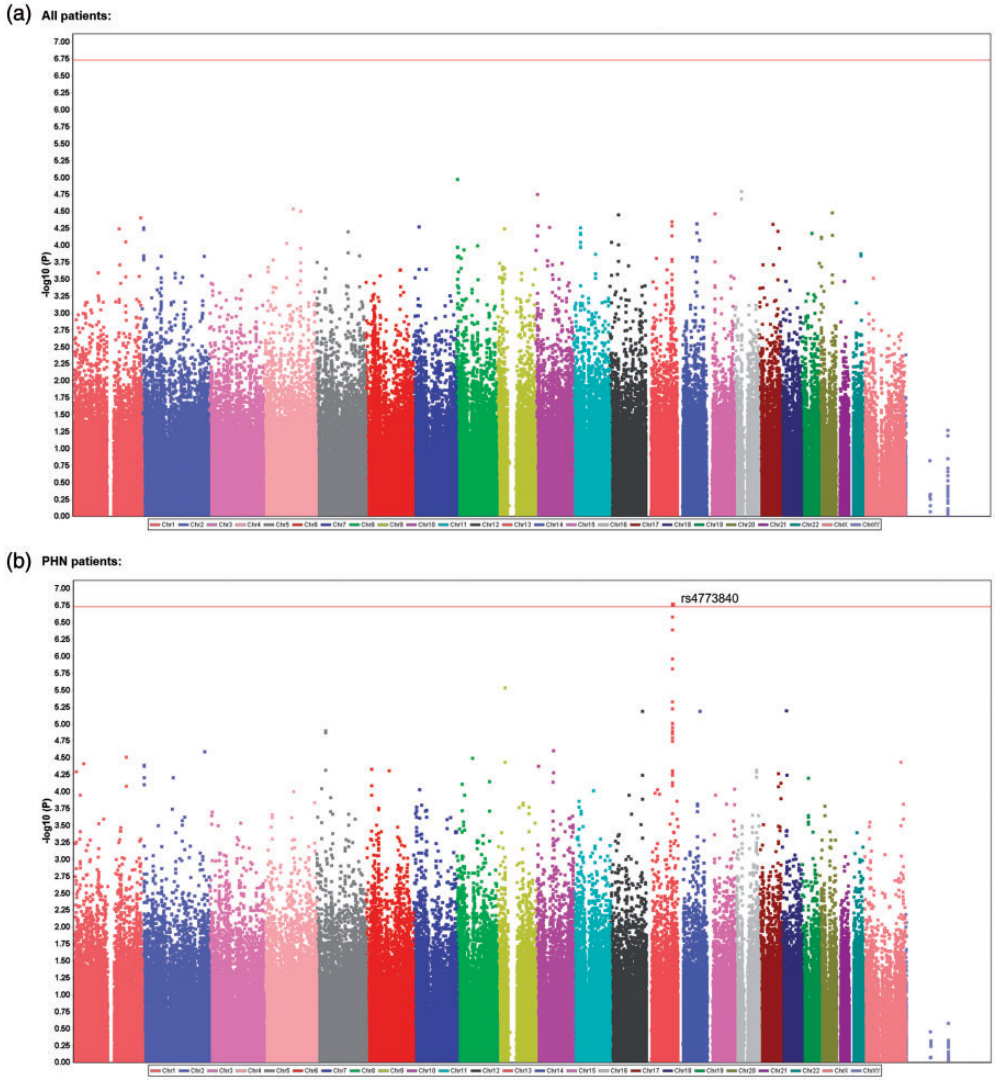

Figure 1. Manhattan plot of the GWAS results. (a) Plot of the analysis of all 191 patients with chronic pain in the trend model. (b) Plot of the analysis that including only patients with PHN. The red line indicates the threshold for a significant association.

patients, *PRKCQ*, was significantly associated with the phenotype (adjusted $p = 0.03722$; Supplementary Table S1, Figure 3(a)). However, none of the genes were significantly associated with the phenotype in any of the genetic models that were used for the analysis of only PHN patients (Supplementary Table S2, Figure 3 (b)). The association between PHN and the *ABCC4* gene, for which the rs4773840 SNP was significantly associated with the phenotype, was only marginally significant in our gene-based analysis (adjusted $p = 0.06364$; Supplementary Table S2, Figure 3(b)). Tables 4 and 5 show the top 20 candidate gene sets that were identified in each genetic model in the gene-set analysis. As a result, the "go_fructose_ metabolic_process" gene set was significantly associated with chronic pain in the recessive model (adjusted $p = 0.003887$; Table 4). Additionally, the "go_regeneration," "go_reactive_ oxygen_species_ metabolic_process," "go_arachidonic_ acid_mono oxygenase_activity," and "go_translation_ regulator_ activity_nucleic_acid_binding" gene sets were significantly associated with PHN in the trend, dominant, and recessive models, respectively (adjusted $p = 0.03587$, 0.04548, 0.004380, and 0.01472, respectively; Table 5). The genes that were included in these gene sets are listed in Supplementary Table S3. The *ABCC4* gene was not included in any of the gene sets; thus, the *PRKCQ* gene was included in the "go_regeneration" gene set (Supplementary Table S3). Among these genes, only three (*PFKFB1*, *APOA4*, and *BCL2*) were commonly included in two kinds of gene sets (Supplementary Table S3).

Table 3. Top 20 candidate SNPs selected from GWAS for patients with postherpetic neuralgia (PHN).

Model	Rank	CHR	SNP	Position	*P*	Related gene	Genotype (patients)			Genotype (controls)		
							A/A	A/B	B/B	A/A	A/B	B/B
Trend	1	13	rs4773840	94568426	0.0000001638*	*ABCC4*	16	40	33	10	96	176
Trend	2	13	rs1678353	94547567	0.000000255	*ABCC4*	17	39	33	9	103	170
Trend	3	13	rs1751057	94548737	0.0000003913	*ABCC4*	17	39	33	10	102	170
Trend	4	13	rs1678395	94563955	0.000001063	*ABCC4*	16	40	33	11	101	170
Trend	5	13	rs1678362	94529692	0.000001482	*ABCC4*	16	41	32	12	103	167
Trend	5	13	rs1751052	94531379	0.000001482	*ABCC4*	16	41	32	12	103	167
Trend	5	13	rs1189438	94532991	0.000001482	*ABCC4*	16	41	32	12	103	167
Trend	8	9	rs10114508	26892593	0.000002803		5	36	46	2	63	214
Trend	9	13	rs1729752	94530363	0.000004509	*ABCC4*	18	39	32	14	108	160
Trend	10	13	rs4148540	94491368	0.000005799	*ABCC4*	13	45	31	16	94	172
Trend	10	13	rs4148540	94491368	0.000005799	*ABCC4*	4	42	43	66	136	80
Trend	12	18	rs12458523	19074726	0.00000617	*CABLES1*	11	43	35	14	82	186
Trend	13	14	rs2167151	78933086	0.000006287	*NRXN3*	6	44	39	6	80	196
Trend	14	12	rs10851014	117614600	0.0000063		16	39	34	12	105	165
Trend	15	13	rs1678387	94515907	0.000009474	*ABCC4*	16	39	34	12	105	165
Trend	15	13	rs1678365	94516981	0.000009474	*ABCC4*	16	39	34	12	105	165
Trend	15	13	rs1189451	94520087	0.000009474	*ABCC4*	16	39	34	12	105	165
Trend	15	13	rs2619312	94521040	0.000009474	*ABCC4*	16	39	34	12	105	165
Trend	15	13	rs1751037	94521559	0.000009474	*ABCC4*	16	39	34	12	105	165
Trend	15	13	rs1189461	94521789	0.000009474	*ABCC4*	16	39	34	12	105	165
Trend	15	13	rs1189464	94523867	0.000009474	*ABCC4*	16	39	34	12	105	165
Dominant	1	6	rs4075048	19275975	0.00001134		0	4	85	4	65	213
Dominant	2	14	rs2167151	78933086	0.00001212	*NRXN3*	11	43	35	14	82	186
Dominant	3	2	rs6718476	6112647	0.00001274		11	38	40	59	166	57
Dominant	3	2	rs2693818	6121959	0.00001274		11	38	40	59	166	57
Dominant	5	13	rs4148540	94491368	0.00001754	*ABCC4*	13	45	31	16	94	172
Dominant	6	6	rs9368038	19298240	0.00001905		0	5	84	5	66	211
Dominant	6	6	rs9350106	19303045	0.00001905		0	5	84	5	66	211
Dominant	8	12	rs10851014	117614600	0.00002548		6	44	39	6	80	196
Dominant	9	2	rs4675047	226665422	0.00002799		3	24	62	29	125	119
Dominant	10	1	rs2176360	188083580	0.00002889		9	50	30	14	100	168
Dominant	11	7	rs4722067	21868091	0.00003014	*DNAH11*	16	33	40	82	140	60
Dominant	12	16	rs12596324	26039779	0.00003039	*HS3ST4*	10	29	50	44	150	88
Dominant	13	6	rs9358193	19281466	0.0000309		0	5	84	5	64	211
Dominant	14	6	rs648248	117187750	0.00003254	*FAM162B*	13	30	46	48	157	77
Dominant	15	9	rs10114508	26892593	0.00003959		5	36	46	2	63	214
Dominant	16	13	rs4773840	94568426	0.00004453	*ABCC4*	16	40	33	10	96	176
Dominant	17	8	rs7822451	17266781	0.00004517	*MTMR7*	5	29	55	35	143	104
Dominant	18	7	rs10278297	135341940	0.00004885		15	33	41	49	168	65

(continued)

Table 3. Continued.

Model	Rank	CHR	SNP	Position	*P*	Related gene	Genotype (patients)			Genotype (controls)		
							A/A	A/B	B/B	A/A	A/B	B/B
Dominant	19	1	rs624912	236807876	0.00005329		7	21	61	32	126	123
Dominant	20	8	rs2658914	56511974	0.00005364	*XKR4*	0	18	71	13	111	158
Recessive	1	13	rs1678353	94547567	0.00000369	*ABCC4*	17	39	33	9	103	170
Recessive	2	13	rs1751057	94548737	0.000008018	*ABCC4*	17	39	33	10	102	170
Recessive	3	18	rs12458523	19074726	0.00001884	*CABLES1*	4	42	43	66	136	80
Recessive	4	13	rs4773840	94568426	0.00002414	*ABCC4*	16	40	33	10	96	176
Recessive	5	12	rs10849659	118331044	0.00002555	*CCDC60*	19	28	42	15	132	135
Recessive	6	13	rs1729752	94530363	0.00003901	*ABCC4*	18	39	32	14	108	160
Recessive	7	2	rs10208470	75356624	0.00004381		2	49	38	52	122	108
Recessive	8	2	rs10205827	75356361	0.00004401		2	49	38	52	122	107
Recessive	9	12	rs4465416	118338125	0.00004502	*CCDC60*	19	28	42	16	131	135
Recessive	10	13	rs9576139	36396944	0.00004547		0	37	52	36	108	138
Recessive	11	13	rs1678395	94563955	0.0000476	*ABCC4*	16	40	33	11	101	170
Recessive	12	12	rs4300442	118324515	0.00005037	*CCDC60*	20	29	40	17	131	134
Recessive	13	2	rs1015802	153792446	0.00005759		8	20	60	1	77	204
Recessive	14	2	rs11680628	153839089	0.00006176		8	20	61	1	75	206
Recessive	15	2	rs1439630	153839620	0.00006204		10	24	55	3	82	197
Recessive	15	2	rs7556698	153850240	0.00006204		10	24	55	3	81	198
Recessive	17	9	rs10981230	113851385	0.00007136	*MIR3134,SUSD1*	35	38	16	50	152	80
Recessive	18	13	rs9557470	100094751	0.00007228	*TMTC4*	23	31	35	24	130	128
Recessive	19	1	rs4129058	5310402	0.00007338		2	59	28	50	131	101
Recessive	20	4	rs7670109	184691188	0.00008034		35	37	17	51	157	74

Model, the genetic model in which candidate SNPs were selected by GWAS; CHR, chromosome number.
Related gene, the nearest gene from the SNP site; A/A, homozygote for the minor allele in each SNP.
A/B, heterozygote for the major allele in each SNP; B/B, homozygote for the major allele in each SNP.
*, Significant association after correction for multiple testing.

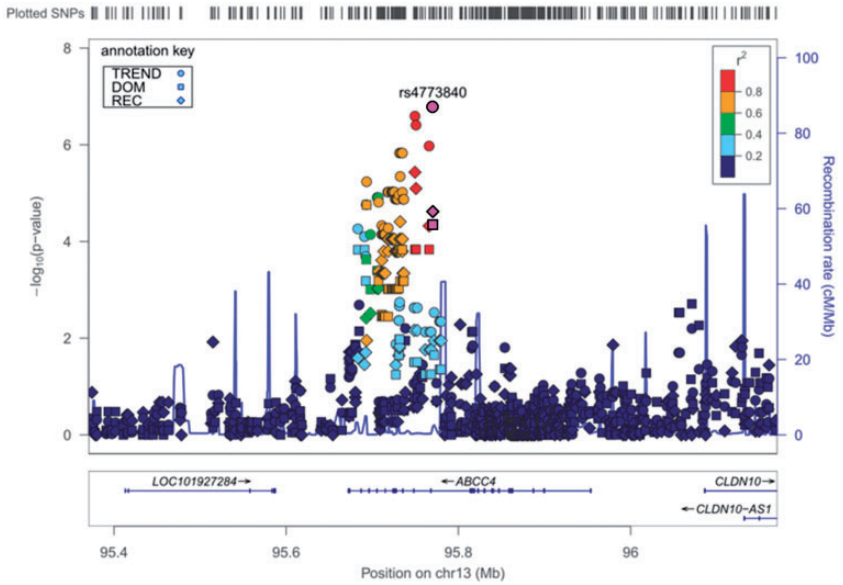

Figure 2. Regional plot of a potent locus that was associated with PHN. The genomic region 400 kbp upstream and downstream of the rs4773840 SNP on chromosome 13 is illustrated. The results of the association analyses in each genetic model were plotted, with the information on annotated genes, estimated recombination rates, and the pairwise-calculated strength of linkage disequilibrium (LD; r^2 values) with the rs4773840 SNP in this region.

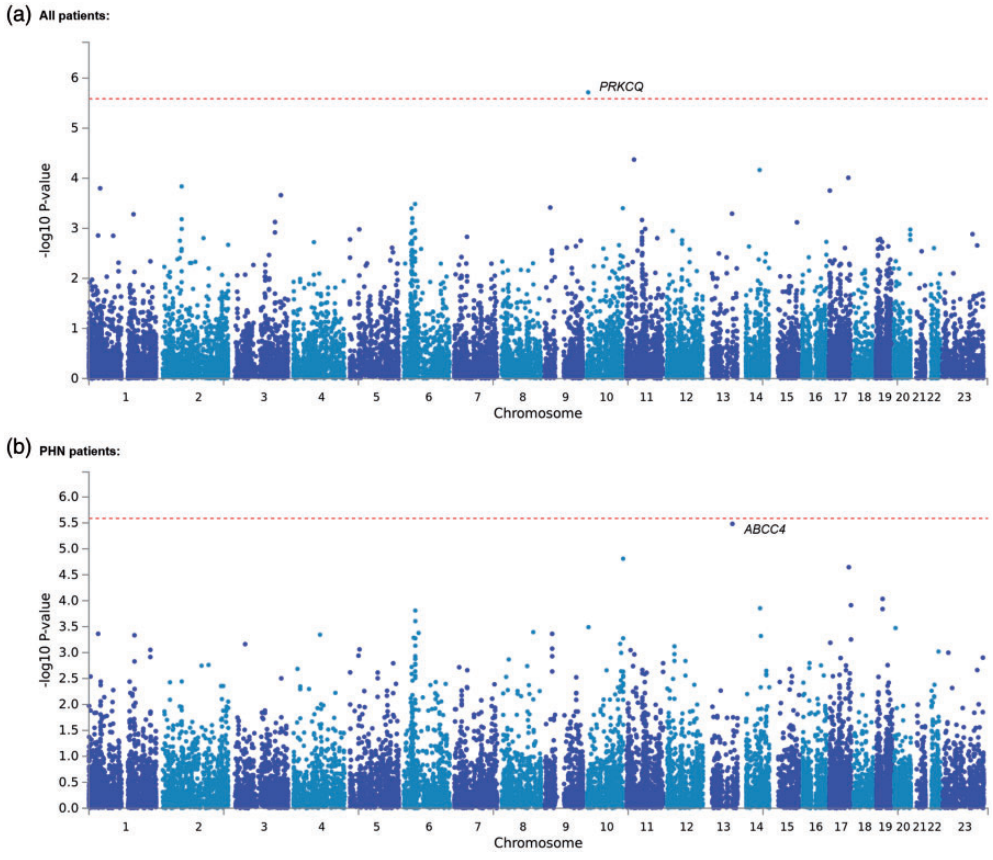

Figure 3. Manhattan plot of the results of the gene-based analyses. (a) Plot of the analysis with all 191 patients with chronic pain in the trend model. (b) Plot of the analysis that included only patients with PHN. The dotted red line indicates the threshold for a significant association.

Table 4. Top 20 candidate gene sets selected from gene-set analysis for all patients.

Model	Rank	Gene set name	nGenes	Beta	SE	*P*	*P*[a]
Trend	1	go_transmembrane_receptor_protein_tyrosine_kinase_signaling_pathway	490	0.14	0.0377	0.00010183	1
Trend	2	go_morphogenesis_of_a_polarized_epithelium	27	0.521	0.146	0.00018275	1
Trend	3	chang_pou5f1_targets_up	15	0.697	0.201	0.00027201	1
Trend	4	pid_fanconi_pathway	46	0.421	0.122	0.00028575	1
Trend	5	go_oxidoreductase_activity_acting_on_paired_donors_with_incorporation_or_reduction_of_molecular_oxygen_reduced_flavin_or_flavoprotein_as_one_donor_and_incorporation_of_one_atom_of_oxygen	24	0.613	0.184	0.00043085	1
Trend	6	go_apical_protein_localization	12	0.825	0.25	0.00048715	1
Trend	7	delaserna_myod_targets_dn	56	0.375	0.115	0.0005732	1
Trend	8	go_execution_phase_of_apoptosis	53	0.379	0.117	0.00059648	1
Trend	9	go_atpase_activity_coupled	299	0.149	0.0462	0.00064486	1
Trend	10	liu_sox4_targets_dn	299	0.152	0.0472	0.00066145	1
Trend	11	firestein_ctnnb1_pathway	32	0.475	0.149	0.00070207	1
Trend	12	ning_chronic_obstructive_pulmonary_disease_dn	117	0.23	0.0722	0.00072694	1
Trend	13	mariadason_response_to_butyrate_curcumin_sulindac_tsa_1	9	1.11	0.349	0.00074306	1
Trend	14	ross_aml_with_pml_rara_fusion	72	0.316	0.1	0.00082081	1
Trend	15	go_establishment_of_tissue_polarity	17	0.57	0.181	0.00083939	1
Trend	16	kondo_colon_cancer_hcp_with_h3k27me1	26	0.521	0.168	0.00098707	1
Trend	17	go_enzyme_linked_receptor_protein_signaling_pathway	675	0.1	0.0327	0.0010865	1
Trend	18	go_atp_dependent_dna_helicase_activity	33	0.411	0.135	0.0011325	1
Trend	19	ikeda_mir30_targets_up	115	0.232	0.0772	0.0013186	1
Trend	20	go_gamma_tubulin_binding	24	0.498	0.166	0.0013693	1
Dominant	1	go_arachidonic_acid_monooxygenase_activity	15	1.14	0.263	0.0000075774	0.08072962
Dominant	2	go_oxidoreductase_activity_acting_on_paired_donors_with_incorporation_or_reduction_of_molecular_oxygen_reduced_flavin_or_flavoprotein_as_one_donor_and_incorporation_of_one_atom_of_oxygen	24	0.75	0.188	0.000032941	0.350953414
Dominant	3	pid_fanconi_pathway	46	0.453	0.125	0.00013871	1
Dominant	4	go_positive_regulation_of_receptor_recycling	11	0.767	0.215	0.00018422	1
Dominant	5	go_dna_double_strand_break_processing	19	0.624	0.175	0.00018853	1
Dominant	6	lenaour_dendritic_cell_maturation_up	111	0.252	0.0754	0.00042167	1
Dominant	7	kondo_colon_cancer_hcp_with_h3k27me1	26	0.574	0.172	0.00042761	1
Dominant	8	go_apical_protein_localization	12	0.842	0.255	0.00048821	1
Dominant	9	reactome_xenobiotics	15	0.874	0.266	0.00051506	1
Dominant	10	delaserna_myod_targets_dn	56	0.379	0.118	0.0006494	1

(continued)

Table 4. Continued.

Model	Rank	Gene set name	nGenes	Beta	SE	*P*	P^a
Dominant	11	go_cytoplasmic_dynein_complex	15	0.62	0.195	0.00072543	1
Dominant	12	go_execution_phase_of_apoptosis	53	0.373	0.12	0.0008959	1
Dominant	13	go_cellular_response_to_exogenous_dsrna	12	0.79	0.253	0.00091276	1
Dominant	14	jechlinger_epithelial_to_mesenchymal_transition_up	69	0.315	0.101	0.0009394	1
Dominant	15	go_dna_metabolic_process	728	0.0982	0.0317	0.00098413	1
Dominant	16	taylor_methylated_in_acute_lymphoblastic_leukemia	72	0.306	0.099	0.00099275	1
Dominant	17	reactome_heparan_sulfate_heparin_hs_gag_metabolism	52	0.385	0.126	0.0011419	1
Dominant	18	go_dna_repair	461	0.119	0.0391	0.0011488	1
Dominant	19	go_poly_a_binding	13	0.58	0.19	0.0011566	1
Dominant	20	go_asymmetric_protein_localization	19	0.594	0.195	0.0011843	1
Recessive	1	go_fructose_metabolic_process	14	1.24	0.25	0.00000036488	0.00388743152*
Recessive	2	kang_immortalized_by_tert_up	86	0.349	0.0873	0.000032117	0.342174518
Recessive	3	go_translation_factor_activity_rna_binding	79	0.363	0.0956	0.000072849	0.776133246
Recessive	4	go_regulation_of_hexokinase_activity	11	0.886	0.238	0.00010025	1
Recessive	5	haddad_t_lymphocyte_and_nk_progenitor_up	75	0.344	0.0928	0.00010685	1
Recessive	6	go_regulation_of_attachment_of_spindle_microtubules_to_kinetochore	11	1.04	0.296	0.00022662	1
Recessive	7	go_regulation_of_cell_projection_assembly	148	0.247	0.0703	0.00022671	1
Recessive	8	go_regulation_of_t_cell_tolerance_induction	12	0.712	0.215	0.00045907	1
Recessive	9	zwang_down_by_2nd_egf_pulse	217	0.186	0.0564	0.00049442	1
Recessive	10	go_regulation_of_membrane_lipid_metabolic_process	13	0.782	0.238	0.00051065	1
Recessive	11	kenny_ctnnb1_targets_up	50	0.396	0.122	0.00056843	1
Recessive	12	go_immunoglobulin_binding	18	0.581	0.182	0.00068753	1
Recessive	13	reactome_tca_cycle_and_respiratory_electron_transport	115	0.26	0.0822	0.00076392	1
Recessive	14	nielsen_synovial_sarcoma_dn	19	0.791	0.25	0.00076547	1
Recessive	15	doane_breast_cancer_esr1_dn	48	0.376	0.119	0.00078823	1
Recessive	16	go_dna_replication_dependent_nucleosome_organization	31	0.839	0.267	0.0008361	1
Recessive	17	go_t_cell_apoptotic_process	15	0.651	0.207	0.00084519	1
Recessive	18	go_lymphocyte_apoptotic_process	18	0.605	0.193	0.0008619	1
Recessive	19	go_regulation_of_pseudopodium_assembly	13	0.735	0.237	0.00098328	1
Recessive	20	lee_aging_cerebellum_dn	80	0.292	0.0946	0.0010161	1

Model, the genetic model in which candidate gene sets were selected by analysis; nGenes, the number of genes in the data that are in the gene set; Beta, the regression coefficient of the gene set; SE, the standard error of the regression coefficient; P^a, adjusted P-value for multiple testing; *, Significant association after the conservative Bonferroni correction.

Table 5. Top 20 candidate gene sets selected from gene-set analysis for patients with postherpetic neuralgia (PHN).

Model	Rank	Gene set name	nGenes	Beta	SE	*P*	*P*[a]
Trend	1	go_regeneration	153	0.308	0.0685	0.0000033672	0.0358741488*
Trend	2	go_reactive_oxygen_species_metabolic_process	92	0.411	0.0922	0.0000042685	0.045476599*
Trend	3	go_organ_regeneration	79	0.355	0.0966	0.00011875	1
Trend	4	reactome_p2y_receptors	12	1.03	0.282	0.0001333	1
Trend	5	tuomisto_tumor_suppression_by_col13a1_up	16	0.771	0.215	0.00016802	1
Trend	6	go_regulation_of_mrna_3_end_processing	27	0.494	0.141	0.00023174	1
Trend	7	go_au_rich_element_binding	21	0.655	0.192	0.00032672	1
Trend	8	go_regulation_of_nuclear_transcribed_mrna_poly_a_tail_shortening	11	0.765	0.226	0.00035697	1
Trend	9	go_rna_destabilization	16	0.601	0.178	0.00037747	1
Trend	10	go_apical_protein_localization	12	0.826	0.251	0.00050398	1
Trend	11	murakami_uv_response_6hr_dn	19	0.637	0.195	0.00053107	1
Trend	12	go_superoxide_metabolic_process	30	0.623	0.19	0.00053228	1
Trend	13	go_negative_regulation_of_cellular_response_to_insulin_stimulus	31	0.513	0.159	0.00060983	1
Trend	14	go_execution_phase_of_apoptosis	53	0.375	0.117	0.00068738	1
Trend	15	hernandez_aberrant_mitosis_by_docetacel_4nm_up	21	0.624	0.196	0.00072487	1
Trend	16	go_regulation_of_mrna_polyadenylation	10	0.629	0.198	0.00074015	1
Trend	17	pid_nfat_tfpathway	47	0.401	0.13	0.00099145	1
Trend	18	go_regulation_of_transferase_activity	920	0.0867	0.0283	0.0010735	1
Trend	19	go_axon	411	0.125	0.0413	0.0012388	1
Trend	20	go_regulation_of_cellular_amide_metabolic_process	344	0.136	0.0449	0.0012506	1
Dominant	1	go_arachidonic_acid_monooxygenase_activity	15	1.33	0.269	0.00000041113	0.00438017902*
Dominant	2	reactome_p2y_receptors	12	1.23	0.294	0.000015196	0.161898184
Dominant	3	go_regulation_of_mrna_polyadenylation	10	0.791	0.206	0.000061699	0.657341146
Dominant	4	go_regulation_of_mrna_3_end_processing	27	0.551	0.147	0.000088695	0.94495653
Dominant	5	go_long_chain_fatty_acid_metabolic_process	87	0.342	0.0913	0.000090289	0.961939006
Dominant	6	go_negative_regulation_of_binding	127	0.273	0.074	0.00011268	1
Dominant	7	go_neuron_apoptotic_process	34	0.522	0.143	0.0001309	1
Dominant	8	go_reactive_oxygen_species_metabolic_process	92	0.347	0.0961	0.00015146	1
Dominant	9	murakami_uv_response_6hr_dn	19	0.724	0.203	0.00017847	1
Dominant	10	graham_normal_quiescent_vs_normal_dividing_up	64	0.433	0.122	0.00019318	1
Dominant	11	go_regeneration	153	0.252	0.0713	0.000204	1
Dominant	12	reactome_signaling_by_notch4	12	0.933	0.264	0.00020967	1
Dominant	13	tuomisto_tumor_suppression_by_col13a1_up	16	0.772	0.224	0.00028268	1
Dominant	14	go_arachidonic_acid_metabolic_process	50	0.424	0.126	0.00036618	1
Dominant	15	go_rna_destabilization	16	0.626	0.186	0.00038011	1
Dominant	16	17	0.667	0.2	0.00041978	1	
Dominant	17		31	0.548	0.165	0.00044684	1

(continued)

Table 5. Continued.

Model	Rank	Gene set name	nGenes	Beta	SE	*P*	P^a
		go_negative_regulation_of_cellular_response_to_insulin_stimulus					
Dominant	18	reactome_xenobiotics	15	0.868	0.273	0.00073068	1
Dominant	19	go_apical_protein_localization	12	0.83	0.262	0.00075723	1
Dominant	20	go_neuron_death	46	0.4	0.127	0.00080182	1
Recessive	1	go_translation_regulator_activity_nucleic_acid_binding	17	1.06	0.226	0.0000013818	0.0147216972*
Recessive	2	galluzzi_permeabilize_mitochondria	41	0.546	0.13	0.000014119	0.150423826
Recessive	3	go_fructose_metabolic_process	14	1.06	0.258	0.000020033	0.213431582
Recessive	4	go_regulation_of_hexokinase_activity	11	0.995	0.253	0.000043055	0.45870797
Recessive	5	go_immunoglobulin_binding	18	0.719	0.191	0.000084426	0.899474604
Recessive	6	go_heat_shock_protein_binding	88	0.329	0.0876	0.000088017	0.937733118
Recessive	7	go_peptide_antigen_binding	25	0.795	0.216	0.00011626	1
Recessive	8	go_ikappab_kinase_complex	11	1.02	0.282	0.0001527	1
Recessive	9	mootha_glycolysis	21	0.771	0.215	0.00016298	1
Recessive	10	kang_immortalized_by_tert_up	86	0.318	0.0922	0.00028655	1
Recessive	11	bogni_treatment_related_myeloid_leukemia_up	29	0.553	0.163	0.00033607	1
Recessive	12	go_igg_binding	7	0.947	0.281	0.00037687	1
Recessive	13	ellwood_myc_targets_up	13	0.839	0.249	0.00038154	1
Recessive	14	dorsam_hoxa9_targets_up	35	0.449	0.138	0.0005898	1
Recessive	15	reactome_abortive_elongation_of_hiv1_transcript_in_the_absence_of_tat	23	0.64	0.201	0.00073176	1
Recessive	16	krieg_hypoxia_not_via_kdm3a	716	0.109	0.0343	0.00073919	1
Recessive	17	go_central_nervous_system_development	841	0.0994	0.0316	0.00084853	1
Recessive	18	shin_b_cell_lymphoma_cluster_9	19	0.659	0.212	0.0009452	1
Recessive	19	go_regulation_of_protein_sumoylation	21	0.596	0.192	0.00094559	1
Recessive	20	holleman_daunorubicin_b_all_up	10	1.16	0.374	0.00097434	1

Model, the genetic model in which candidate gene sets were selected by analysis; nGenes, the number of genes in the data that are in the gene set; Beta, the regression coefficient of the gene set; SE, the standard error of the regression coefficient; P^a, adjusted P-value for multiple testing.

*Significant association after the conservative Bonferroni correction.

Identification of genetic polymorphisms associated with the effects of drugs for the treatment of pain in patients

Various types of drugs were administered to the patients for the treatment of pain. Although some of these drugs were effective for some patients, others were not. We performed another GWAS of 191 patient subjects to explore SNPs that were associated with the efficacy of these drugs, which were divided into major five groups (opioids, antidepressants, anticonvulsants, NSAIDs, and GABA receptor agonists; Table 1). Supplementary Tables S4 to S8 show the top 20 candidates for these drugs in each genetic model. However, none of the SNPs were genome-wide significantly associated with the phenotypes ($p \geq 1.858 \times 10^{-7}$; Supplementary Tables S4–S8). The best candidate SNPs with the lowest p values were rs7811258 SNP in the dominant model for opioids (nominal $p = 1.655 \times 10^{-6}$; Supplementary Table S4), rs10793705 SNP in the trend model for antidepressants (nominal $p = 1.714 \times 10^{-6}$; Supplementary Table S5), rs2300525 SNP in the dominant model for anticonvulsants (nominal $p = 1.403 \times 10^{-6}$; Supplementary Table S6), rs2195962 and rs12461406 SNPs in the dominant model for NSAIDs (nominal $p = 3.573 \times 10^{-6}$; Supplementary Table S7), and rs7094057 SNP in the trend model for GABA receptor agonists (nominal $p = 3.311 \times 10^{-6}$; Supplementary Table S8).

Discussion

To identify potential genetic variants that contribute to the susceptibility to chronic pain conditions and the effects of several types of drugs that are used to treat pain, we conducted an overall GWAS of patients with chronic pain and control subjects. We also explored genetic factors that are associated with PHN by performing another GWAS. The results suggested that carriers of the C-allele of the rs4773840 SNP within the *ABCC4* gene region were more susceptible to PHN (Table 3), and several SNPs within or around the *PRKCQ* gene region jointly influenced the risk of developing chronic pain conditions. Furthermore, we found several gene sets that were possibly associated with these phenotypes. Meanwhile, we found no SNPs that were significantly associated with the efficacy of drugs for the treatment of pain. One of the reasons for this lack of an association might be related to the small sample size for each association analysis for each drug, which resulted in a lack of statistical power to detect positive associations. Indeed, the largest number of samples was only 99 in the analysis of anticonvulsant drugs among five major types of drugs (Table 1), whereas the total number of patients with chronic pain who were recruited in the study was 194, indicating that less than half of the patients were included in these analyses. Future studies with larger sample sizes will clarify which SNPs affect the efficacy of drugs to treat chronic pain.

Chronic pain is a common and heterogenous clinical condition. Previous studies have mostly explored genetic factors that are associated with chronic pain in a particular subset of patients, such as patients with CWP,[13,15,28] CPSP,[22] chronic back pain,[43] and neuropathic pain, including diabetic neuropathic pain.[23–25,29–32] The disease status of the patients in our samples was diverse, and the sample size for each disease status was fairly small (Table 1), thus hampering genetic association analyses of each patient subgroup, with the exception of patients with PHN. Therefore, the present study conducted analyses of overall patients with chronic pain and a subgroup of patients with PHN. Although the analysis of overall patients might present a risk that the genetic effects on each phenotype are obscured or not precisely detected, one could assume that some genetic factors that commonly affect chronic pain can be detected among all of the genetic factors. Postherpetic neuralgia is a neuropathic pain disorder that occurs most often in the elderly and is a major complication of herpes zoster, with spontaneous pain and stimulus-evoked pain, such as allodynia and hyperpathia.[44–47] The genetic factors that contribute to PHN are poorly understood. Only a few studies have reported genetic variations that are associated with the susceptibility to PHN, including the human histocompatibility leukocyte antigen (HLA) locus, in which the HLA-A*3303, -B*4403, and -DRB1*1302 alleles have been shown to be associated with the risk of PHN.[47–50] Although the present study did not investigate the HLA locus in detail because of an inability to precisely genotype HLA alleles using commercially available SNP arrays, we comprehensively explored genetic risk factors for PHN at the genome-wide level for the first time, which resulted in the identification of possibly associated SNPs, such as rs4773840 (Table 3).

The best candidate SNP with the lowest p value among the candidate SNPs for PHN was rs4773840, which is located in the intronic region of the *ABCC4* gene on chromosome 13. The *ABCC4* gene encodes the ABCC4 protein, which is a member of the MRP subfamily (MRP4) that is involved in multi-drug resistance and acts as an independent regulator of intracellular cyclic nucleotide levels and mediator of cyclic adenosine monophosphate (cAMP)-dependent signal transduction to the nucleus.[51] The mRNA of this gene was reported to be widely expressed in humans, with particularly high levels in the prostate, but it is barely detectable in the liver.[52] ABCC4 has been implicated in the transport of antiviral agents, anticancer drugs,[53–55] and endogenous molecules, such as prostaglandins, steroids, bile acids,

cyclic nucleotides, and folate.[56–60] Indeed, ABCC4 is involved in the efflux of prostaglandin F2α, and the *ABCC4* gene is reportedly upregulated in ovarian endometriosis tissue compared with normal endometrium tissue,[61] which would be a mechanism that underlies endometriosis, a chronic inflammatory disease that often involves severe pain or infertility.[62,63] The disruption of cAMP and prostaglandin E2 transport by *mrp4* deficiency in mice altered cAMP-mediated signaling and the nociceptive response.[64] These studies suggest that ABCC4 may be involved in some pain-related conditions in humans and mice. To date, many genetic variations within or around the *ABCC4* gene have been identified and characterized in Japanese and other ethnically diverse populations.[65,66] The functional impact of these variations, especially nonsynonymous polymorphisms, have been investigated in previous studies.[67–71] In genetic association studies of disease status and symptoms, SNPs or copy number variations within or around the *ABCC4* gene have been shown to be associated with airway inflammation in asthmatic individuals,[68] unfavorable clinical outcomes in children with acute lymphoblastic leukemia,[69] patients with esophageal squamous cell carcinoma,[72] patients with chemotherapy-induced peripheral neuropathy,[73] and measures of pain symptoms in patients with lung cancer and acute post-radiotherapy pain.[74,75] However, none of these studies included the rs4773840 SNP or other SNPs that were in relatively strong LD with this SNP in our samples ($r^2 \geq 0.8$; Supplementary Figure S3). According to the Genotype-Tissue Expression (GTEx) portal (accessed July 10, 2019; Supplementary Methods), one of the SNPs that is in relatively strong LD with the rs4773840 SNP, rs2950957 (Supplementary Figure S3), significantly affects mRNA expression of the *ABCC4* gene in the muscularis in the human esophagus. Single-nucleotide polymorphisms that are in relatively strong LD with the rs4773840 SNP include two synonymous SNPs in the coding region, rs1189466 and rs1678339 (Supplementary Figure S3), based on the Exome Aggregation Consortium (ExAC) Browser (accessed July 10, 2019; Supplementary Methods). When these SNPs were referred to SNPinfo Web Server and SNPnexus (accessed July 10, 2019; Supplementary Methods), they were predicted to affect splicing as exonic splicing enhancers or exonic splicing silencers, and the rs1678339 SNP was found to be within a putative transcription factor binding site in mice and humans. These results suggest that expression or splicing of the *ABCC4* gene could be affected by the rs4773840 SNP and other SNPs that are in relatively strong LD with this SNP, which might be related to a mechanism that contributes to PHN.

In the gene-based analysis of all patients, the *PRKCQ* gene was significantly associated with the phenotype (Supplementary Table S1; Figure 3(a)). The *PRKCQ* gene encodes protein kinase Cθ (PKCθ), which is a family of serine- and threonine-specific protein kinases. The PRKCQ protein is a calcium-independent and phospholipid-dependent kinase that is important for T-cell activation and highly expressed in the thyroid and lymph nodes.[76,77] Lidocaine, which is used as a local anesthetic, was shown to modulate inflammation in septic patients by decreasing chemokine-induced neutrophil arrest and transendothelial migration by inhibiting PKCθ activation.[78] The PKC inhibitor tamoxifen suppressed paclitaxel-, vincristine-, and bortezomib-induced cold and mechanical allodynia in mice,[79] although the specific role of PKCθ was not clearly revealed in this study. In genetic association studies of disease status and symptoms, SNPs within or around the *PRKCQ* gene were shown to be associated with type 1 diabetes[80] and Crohn's disease,[81,82] both of which may involve symptoms of neuropathy or pain as complications. Significant associations were found between Crohn's disease and the nonsynonymous rs2236379 SNP.[81,82] This SNP was found to be in relatively strong LD with the rs2026432 SNP in our samples according to the SNPinfo Web Server ($r^2 \geq 0.8$), which was among the top 20 candidate SNPs in the present study (Table 2). One of these SNPs may influence the susceptibility to both Crohn's disease and chronic pain partly through the same mechanism, but future studies are required to confirm such a possibility. In the gene-set analysis, several significant associations were also found (Tables 4 and 5). Among the three genes that were commonly included in the two candidate gene sets (Supplementary Table S3), the *BCL2* gene was reported to be upregulated in human cultured cells by capsaicin treatment,[83] which is known to affect inflammatory and pain pathways. However, the precise roles of the gene sets in chronic pain and PHN that were identified in the present study remain unknown and require further investigation.

A major limitation of this study would be the limited sample size. However, some of the previous GWAS have successfully identified SNPs significantly associated with the phenotypes examined in considerably small number of samples (i.e., approximately 200 or less samples).[84,85] Moreover, stronger associations can be found in suitably stratified samples with homogenous property (i.e., diagnosis of PHN) than those in entire number of samples, even if such strong associations may be masked before stratification, as demonstrated in previous studies.[86–89] Nevertheless, further studies will be warranted for replication of the results shown in the present study.

In conclusion, our GWASs identified several SNPs and genes associated with chronic pain and PHN, including the *ABCC4* rs4773840 SNP and *PRKCQ* gene. The present findings require corroboration in future studies with larger sample sizes.

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Author Contributions

DN, SK, MH, and KI conceived and designed the experiments. DN and JH performed the experiments. DN analyzed the data. DN and JH contributed reagents/materials/analysis tools. DN and KI wrote the paper. DN, AH, and KI collected DNA. MI, HA, KH, CY, JK, and SO collected clinical data and DNA.

Declaration of Conflicting Interests

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Supplemental material

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