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Original Article

The association between genome-wide polymorphisms and chronic postoperative pain: a prospective observational study

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Summary

Chronic postoperative pain is common and can have a negative impact on quality of life. Recent studies show that genetic risk factors are likely to play a role, although only gene-targeted analysis has been used to date. This is the first genome-wide association study to identify single-nucleotide polymorphisms associated with the development of chronic postoperative pain based on two independent cohorts. In a discovery cohort, 330 women scheduled for hysterectomy were genotyped. A case–control association analysis compared patients without chronic postoperative pain and the 34 who had severe chronic postoperative pain 3 months after surgery. No single-nucleotide polymorphisms reached genome-wide significance, but several showed suggestive associations with chronic postoperative pain ($p < 1 \times 10^{-5}$). Single-nucleotide polymorphisms with significance $p < 1 \times 10^{-5}$ were followed up in a replication cohort consisting of 203 men and women scheduled for orthopaedic or abdominal surgery. Ten of these patients developed severe chronic postoperative pain. A single-nucleotide polymorphism in *NAV3* was significantly replicated with chronic postoperative pain in the replication cohort ($p = 0.009$). Meta-analysis revealed that two loci (*IQGAP1* and *CRTC3)* were significantly associated with chronic postoperative pain at 3 months (*IQGAP1* $p = 3.93 \times 10^{-6}$ $\beta = 2.3863$, *CRTC3* $p = 2.26 \times 10^{-6}$, $\beta = 2.4209$). The present genome-wide association study provides initial evidence for genetic risk factors of chronic postoperative pain and supports follow-up studies.

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Introduction

Moderate to severe chronic postoperative pain is a debilitating condition affecting between 5% and 85% of patients undergoing surgery and has a large negative impact on the quality of life (QoL) and socio-economic status [1–3]. It is defined as "*pain developed or increased after a surgical procedure, which is present for at least three months, and affecting the QoL*" [4, 5]. Furthermore, the pain is localised to the surgical field or projected innervation area of a nerve and other causes for the pain must have been excluded [4, 6]. Both clinical (e.g. type of surgery) and baseline characteristic (e.g. psychosocial status) risk factors have been described but these do not explain all the observed variance. So far, a good understanding of genetic risk factors for chronic postoperative pain is still lacking [1, 7].

The underlying biology of chronic postoperative pain and genetic heritability is complex and not yet fully understood [8]. However, twin studies in pelvic and low back pain have indicated the importance of genetics in chronic pain phenotypes with an estimated heritability of 40% [9, 10]. Association studies focusing on candidate genes have suggested a possible role for *COMT, GCH1,* and *KCNS1* among others [11–13], but a recent systematic review showed conflicting results for all the genes tested [14]. We tried to identify genetic polymorphisms in the human genome associated with chronic postoperative pain 3 months after surgery by performing a genome-wide association study using a unique discovery cohort of patients undergoing hysterectomy [15]. The advantage of this cohort is the homogenous population consisting of only women (age 18–65, malignancies excluded) [16, 17]. Potential candidates were then further explored in a replication cohort of orthopaedic and abdominal surgeries to verify our findings. Combining different clinical cohorts allows testing for different genetic risk factors associated with chronic postoperative pain in general and each individual cohort gives information on surgery-specific risk factors, as shown before in migraine research [18]. A meta-analysis of the discovery and the replication cohort was conducted to investigate the combined effects of both studies.

Methods

This study was approved by our local Medical Ethical Committee (both discovery and replication study) and all participants gave written informed consent.

An elaborate description of recruitment and data collection protocols for the discovery cohort has been published elsewhere [15]. In brief, a multicentre prospective cohort study was conducted, recruiting patients from four hospitals in the Netherlands. Patients undergoing hysterectomy for benign indications between September 2010 and January 2014 were included in the study. Inclusion criteria were: age between 18 and 65 y; fluency in the Dutch language; elective surgery; and total or subtotal hysterectomy with or without oophorectomy using all types of surgical approach. Exclusion criteria consisted of: illiteracy; history of cancer; and cognitive impairment. Furthermore, patients who reported a malignancy or underwent another surgical procedure during the first postoperative year were not analysed**.** Peripheral blood samples were collected before hysterectomy and genomic DNA was isolated at the clinical genetics department of Maastricht University Medical Center.

For the replication study, two multicentre prospective cohort studies were conducted recruiting patients from three hospitals in Italy. In both studies, peripheral blood samples were collected during surgery and genomic DNA was isolated. Inclusion criteria of the orthopaedic cohort consisted of adult patients of all sexes undergoing total knee arthroplasty with ASA physical status 1–3. All patients enrolled had the same regional anaesthesia procedure in order to reduce any bias related to anaesthesia treatment and difference in intra-operative pain. Patients were excluded if there were contra-indications to regional anaesthesia, unstable neurological diseases, diabetes or pre-surgical pain and if regional anaesthesia block was not effective.

The protocol of the abdominal surgery trial has been already published [19]. Inclusion of the abdominal surgery cohort comprised adult patients of all sexes scheduled for major abdominal or urological surgery without regional anaesthesia, ASA physical status 1–3 and HIV negative. Exclusion criteria consisted of: previous regular use of opioids; history of alcohol/drug abuse; postoperative hospitalisation with sedation/ventilation; severe renal or hepatic impairment; cardiac, neurological and cognitive disorders; abnormal coagulation; low platelet count; BMI $> 30\ kg.m^{-2}$, allergy to the drugs studied, diabetes and pre-surgical pain.

Samples were genotyped at the Department of Genomics at the Life and Brain Center, University of Bonn using the Illumina PsychArray (Infinium PsychArray-24 v1.2 Bead Chip, Illumina Inc., San Diego, (CA, USA)) which contains enrichment in genetic variants associated with psychiatric conditions. A strong psychological component is present in pain and several genes in psychiatric disorders have been associated with chronic postoperative pain as

well as identified through the Pain Genes database (e.g. *COMT* and *OPRM1*) [20–22]. Psychological predictors are an important risk factor for chronic postoperative pain [15, 23, 24]. We believe this approach offers greater potential to identify genetic variants with phenotypic effects in chronic postoperative pain. The array includes 265,000 proven tag single-nucleotide polymorphisms (SNPs) found on the Infinium Core-24 Bead Chip, 245,000 markers from the Infinium Exome-24 Bead Chip and 50,000 additional markers associated with common psychiatric disorders.

Genotypes were called using BeadStudio (Genome Studio v2011.1, Illumina San Diego (CA,USA)). Basic quality control was done using Plink (Plink-1.9) [25, 26]. The quality control parameters consisted of: SNP call rate < 0.95; subject call rate of < 0.95; deviation of Hardy–Weinberg equilibrium ($p < 1 \times 10^{-6}$); and removal of rare variants with a minor allele frequency < 0.01. Heterozygosity of the subjects was tested and outliers (± 3 SD from the mean heterozygosity rate) were removed (see also Supporting Information Fig. S1). No relatedness and sex inconsistencies were found within our cohort. After basic quality control, all AT or CG SNPs were removed from the SNP set. After these control steps, the SNPs were pruned to remove SNPs in linkage disequilibrium ($R^2 > 0.2$), principal components analysis was performed using the Aberrant Package in R, and the first two principal components were analysed for outliers. Together with the principal component analysis, the ancestry and ethnicity of the subjects was determined using HapMap data (as described by ENIGMA) (Fig. 2) [27]. After quality control, the data were prepared for imputation by checking for flipped strands, allele assignments and position of SNPs and converted into variant call format files.

Genotype imputation was performed using the imputation stepwise approach implemented in Minimac3 (https://genome.sph.umich.edu/wiki/Minimac3, University of Michigan, Ann Arbor, MI, USA) and Eagle2 (https://data.broadinstitute.org/alkesgroup/Eagle,v2.3, Broad Institute, Cambridge, MA, USA) using default parameters with European HRC reference panel (http://www.haplotype-reference-consortium.org, version r1.1 2016) [28–30]. Quality control on the imputed dataset was performed with the following parameters: genotype probability > 0.9, imputation accuracy > 0.5, INFO-score > 0.4 and minor allele frequency and genotyping rate was checked again. Regional association plots were made to see the linkage disequilibrium of the significantly associated SNPs and their association with chronic postoperative pain using Locuszoom (http://locuszoom.org, University of Michigan, Ann Arbor, MI, USA) [31].

The primary outcome measured in this cohort was the highest surgery-related pain score at rest during the last week at 3 months after surgery measured by the numeric rating scale (NRS) [15]. Based on the primary outcome measure, patients were divided into a non-pain (NRS = 0) and a chronic postoperative pain (NRS > 3) group to perform an extreme phenotype analysis to increase the power. Patients with mild pain (NRS between 1 and 3) score were not included in the genetic analysis. The gene dosages were tested for an association with chronic postoperative pain using an additive logistic regression model. To decrease false-positives, the maximum threshold of λ was set at 1.08 [32]. Genome-wide significance was set at $p < 5 \times 10^{-8}$ and the analysis was run using SNPTEST (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html, v2.5.4, Oxford University, Oxford, UK) [32, 33]. Single-nucleotide polymorphisms with a p value $< 1 \times 10^{-5}$ were selected as suggestive association SNPs for replication [34]. The same statistical tests were used for the discovery and the replication cohort. Next, the discovery and the replication cohort's association SNP results were used to perform a random-effects meta-analysis in Plink. As the study design of the two cohorts was different, and some covariates (e.g. age) differed significantly between the cohorts, we chose to perform a random-effects meta-analysis. Bonferroni correction was applied to account for multiple testing.

Power calculation for this study was redundant conducted and based on the primary aim of the study and not for the genetic testing, which was a secondary aim. Therefore, this study is underpowered for a genome-wide analysis study and, as a consequence, there is a risk of a type-1 error which should be taken into account when interpreting the results [15].

Results

Figure 1 shows the flow chart for inclusion, follow-up and genetic quality control, which is an extended version of the flow chart of our previous publication [15]. After quality control, samples from 330 patients were available for genetic analysis. Baseline pain and peri-operative characteristics can be found in Table 1. Out of the 330 patients included in the analyses, 269 (81.5%) reported no pain (NRS = 0) related to the hysterectomy at the 3 month follow-up, 27 (8.1%) reported mild pain (NRS between 1 and 3) and 34 (10.3%) reported moderate to severe pain (NRS > 3).

The replication cohort underwent a variety of orthopaedic and abdominal surgeries (pooled in this cohort). The total cohort consisted of 249 patients of which

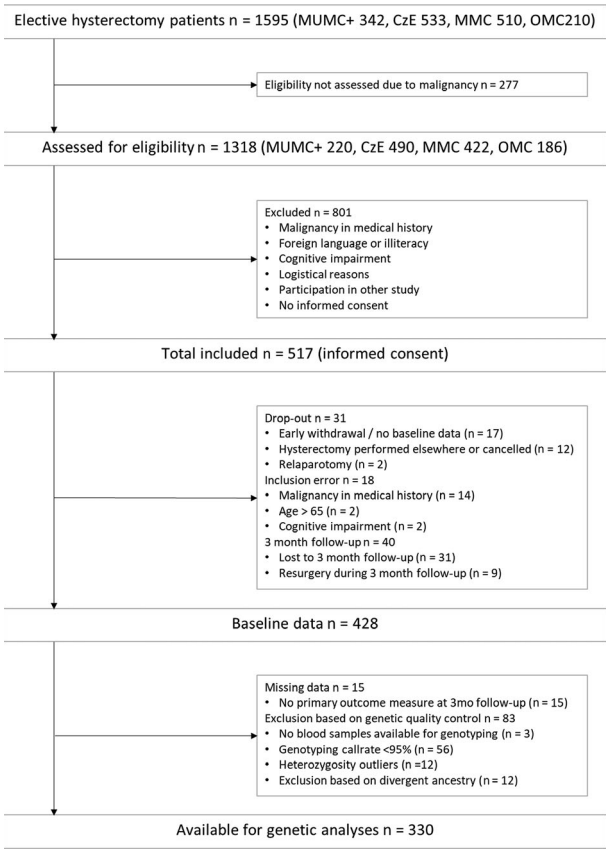

Figure 1 Patient inclusion in the discovery cohort. MUMC+, Maastricht University Medical Center+; CzE, Catharina Hospital Eindhoven; MMC, Máxima Medical Center Veldhoven; OMC, Orbis Medical Center Sittard Geleen; n, sample size.

samples of 203 patients (67 men and 136 women) were available for genetic analysis. Out of these 203 patients, 157 received no intervention and 46 received an infusion of steroids (methylprednisolone) in the first 7 days. A total of 190 patients were available for genetic analysis. It was decided to continue with the women without intervention leading to 106 women included in the study. After quality control, the results of 99 patients were available for genetic analysis. Baseline pain and peri-operative characteristics can be found in Table 1. Out of the 99 patients included in the analyses, 39 (39.4%) reported no pain (NRS = 0) related to the hysterectomy at the 3-month follow-up, 37 (37.4%) reported mild pain (NRS between 1 and 3) and 11 (11.1%) reported moderate to severe pain (NRS > 3).

Ethnicity of both cohorts was checked using the HapMap data and was determined to be of European background (Fig. 2). Subjects deviating from this background were not analysed during quality control (discovery cohorts n = 12, replication cohort n = 4).

After quality control and imputation, 6,293,655 SNPs were included analysis. Overall association results are depicted in Fig. 3a. The QQ plot showed no apparent deviation from the null distribution of p values (Fig. 3b) and the genomic inflation factor (Fig. 3b, $\lambda_{GC} = 1.065$) indicated only a slight inflation of the model without covariates. Although none of the SNPs tested reached the threshold for genome-wide significance ($p < 5 \times 10^{-8}$, Fig 3a), several reached a suggestive level of association (107 SNPs with $p < 1 \times 10^{-5}$, Fig 3a) which were further analysed and labelled as 'SNPs of interest'. Results of the top loci are shown in Table 2 and all SNPs were annotated using GRCh37.p13. A SNP cluster tagged by rs62281806 in *FNDC3B* was the most significant hit ($p = 5.59 \times 10^{-7}$). Other genes tagged by SNPs included *EDNRA, NAV3, TLL2, RSU1, IQGAP1, TMEM63B, PJA2, CRTC3* and *DLG2*. None of these genes have been associated with chronic postoperative pain before, according to a recent systematic review [14]. The detailed results of the discovery cohort (SNPs $p < 0.05$) are available on request from the correspondence author.

In the discovery cohort, no SNP reached the threshold for genome-wide significance. We decided to replicate only those SNPs in the replication cohort which showed a suggestive association ($p < 1 \times 10^{-5}$) with the phenotype to increase the power [34]. The SNPs (n = 107) showing suggestive association in the discovery cohort were further evaluated in an independent replication cohort. Results of the top replication loci are shown in Table 2 and the complete results in the Supporting Information Table S1. The SNP (rs118184265) in *NAV3* showed nominal significance ($p < 0.01$) with chronic postoperative pain at 3 months and two SNPs within *RSU1* (rs7894047, rs7893777) showed a trend towards statistical significance ($p = 0.068$). As the number of patients was limited, we decided to increase the cases by including all women with mild pain at 3 months (NRS > 0). The *NAV3* SNP remained significant although somewhat decreased ($p = 0.014$) and the *RSU1* SNPs became significant ($p = 0.049$) (see also Supporting Information Table S2). The direction of the effect in the replication study is reversed compared with the discovery cohort.

The SNPs studied in both the discovery and the replication cohort (n = 96) were analysed in a random-effects meta-analysis to see the overall effects. Results of the top loci are shown in Table 2 and the complete results in the Supporting Information Table S3. The most significant SNP was rs117119665 in *CRTC3* ($p = 2.26^{-6}$, $\beta = 2.4209$, adjusted $p = 2.41^{-4}$). The second significant SNP was rs1145324 in *IQGAP1* ($p = 3.93^{-6}$, $\beta = 2.3863$, adjusted

Table 1 Baseline and peri-operative patient characteristics of all patients available for genetic analysis. Values are mean (SD) or number (proportion). Missing data are reflected in the proportions.

	Discovery cohort n = 330	**Replication cohort n = 99**
Sex		
Female	330 (100%)	99 (100%)
Age	46.9 (7.2)	69.3 (9.6)
ASA physical status		
1	179 (54.2%)	4 (16.6%)[a]
2	135 (40.9%)	9 (37.5%)[a]
3	4 (1.2%)	6 (25.0%)[a]
Pre-operative pain (surgical indication related)		
NRS 0–3	161 (48.8%)	99 (100%)
NRS 4–10	169 (51.2%)	Patients with pre-surgical pain were excluded
Pain at rest at recovery area (acutely after surgery)[b]		
NRS 0–3	162 (49.1%)	64 (64.6%)
NRS 4–10	133 (40.3%)	31 (31.3%)
Pain on postoperative day 4 (highest NRS last 24 h)		
NRS 0–3	179 (54.2%)	74 (74.7%)
NRS 4–10	111 (33.6%)	6 (6.06%)
Pain at 3 months (highest NRS last week)		
NRS 0–3	296 (89.7%)	76 (76.7%)
NRS 4–10	34 (10.3%)	11 (11.1%)

NRS, numeric rating scale
[a]ASA physical status only available for the patients who underwent abdominal or urological surgery (n = 24).
[b]Pain at recovery area was measured at 1 h after surgery for discovery cohort, 0 h for the abdominal and urological surgeries and after 6 h for orthopaedic surgery.

$p = 4.20^{-4}$). Several nominally significant associations were found (rs11655475 and rs4790802, $p < 0.05$) but these were not annotated to a gene.

Both significant SNPs are located closely together on chromosome 15 within a region of low recombination (Fig. 4). To test whether the two SNPs (rs117119665 and rs1145324) are independent or part of the same locus, we performed a conditional analysis of each SNP with the other SNP as covariate. This analysis showed that the association with chronic postoperative pain disappeared ($p > 0.1$) and, thus, the SNPs are not independent. There is a possible involvement of rs4347600 in *CRTC3* as well but this SNP was not tested in the replication cohort and could, therefore, not be entered into the meta-analysis (Fig. 4).

Discussion

One locus (*NAV3*) of the SNPs of interest was associated with chronic postoperative pain in the replication cohort. Two loci (*CRTC3* and *IQGAP1)* were significantly associated with chronic postoperative pain in the meta-analysis of the discovery and replication cohort. Unfortunately, the sample size of the discovery cohort was too small and, thus, the study was underpowered. The replication cohort and meta-analysis partially overcome this problem but results should be interpreted cautiously. Nevertheless, the study provides an initial insight to genome-wide risk factors of chronic postoperative pain.

The meta-analysis indicated two genes (*CRTC3* and *IQGAP1)* as risk loci for the development of chronic postoperative pain. However, neither has been associated with pain before according to the PainGenesDatabase and the Human Pain Genetics Database [22, 35]. CREB-regulated transcription co-activator 3 (*CRTC3*) is expressed in a variety of tissues including the nervous system, is involved in regulation of CREB-dependent transcription of genes and inhibits adenylyl cyclase in response to catecholamine signalling [36, 37]. It has been associated with several abdominal disorders such as Crohn's and inflammatory bowel disease and cognitive information processing [38–40]. IQ motif containing GTPase-activating protein 1 (*IQGAP1*) is expressed throughout the body including the nervous system, and is involved in cytoskeleton regulation, signalling

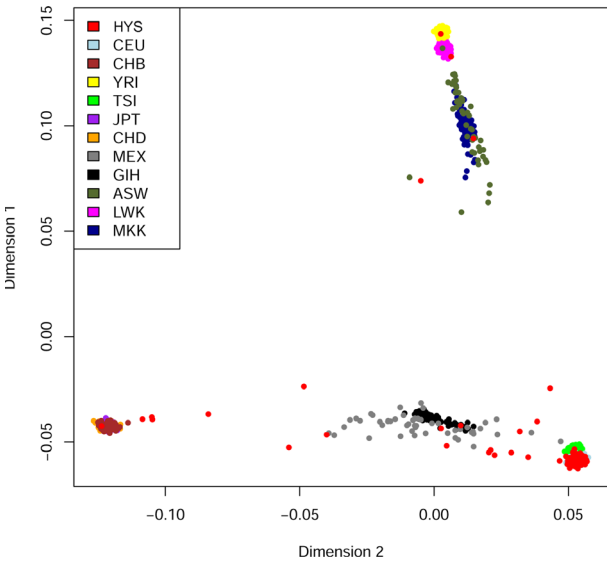

Figure 2 Ancestry plot based on the multidimension scaling method described by ENIGMA and population defined by HapMap. The discovery cohort (HYS) is plotted amongst the populations defined by HapMap. CEU, Utah residents with European ancestry; CHB, Han Chinese from Beijing, China; YRI, Yoruda from Ibadan, Nigeria; TSI, Italian from Tuscany, Italy; JPT, Japanese from Tokyo, Japan; CHD, Chinese from Denver, Colorado, USA; MEX, Mexican ancestry in Los Angeles, California, USA; GIH, Gujarati Indians from Houston, Texas; ASW, African ancestry from Southwest USA; LWK, Luhya from Webuye, Kenya; MKK, Maasai from Kinyawa, Kenya.

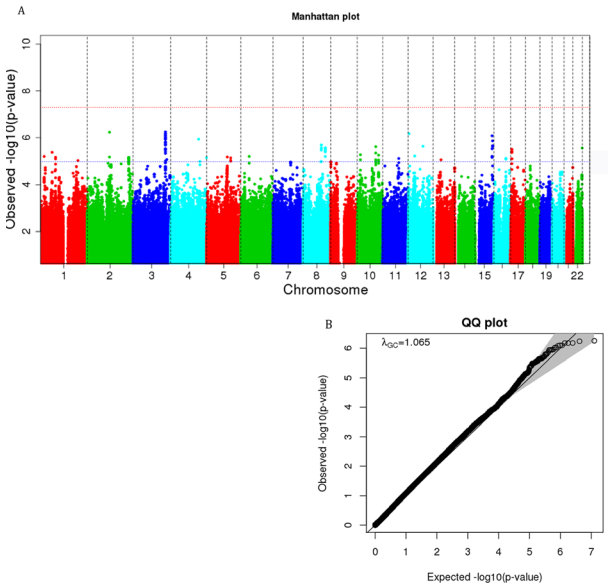

Figure 3 Manhattan plot and quantile-quantile plot representing the results of the genome-wide association analysis of the discovery cohort. (a) Manhattan plot representing the association between single-nucleotide polymorphism (SNP) genotype and chronic postoperative pain at 3 months after surgery in patients with a Caucasian ancestry. The negative $\log_{10}$ p values (y-axis) are plotted against their chromosomal positions (x-axis). The red dotted line indicates the genome-wide significance level ($p > 5.0 \times 10^{-8}$), the blue dotted line indicates the SNPs of interest level ($p > 1.0 \times 10^{-5}$). (b) QQ plot of genotyped and imputed SNPs. Observed p values are plotted against the expected p values. The lambda of 1.065 indicates a small genomic inflation corroborated by the near perfect correlation between the observed and expected p values. Figures were created using the quality control of GWAS data package in R.

molecules and cell motility [36]. It has been associated with immune system functioning and multiple sclerosis [41, 42]. Interestingly, two genome-wide association studies found both *CRTC3* and *IQGAP1* associated with their primary outcome measure (heel bone mineral density and neutrophil percentage of white cells, respectively) indicating a possible synergism or interaction between the two genes [42, 43]. In our study, the SNPs in both genes are in linkage disequilibrium with each other and influence the development of chronic postoperative pain together. Their involvement in the immune system is supportive of postoperative infection being a risk factor for the development of chronic postoperative pain, as previously shown [15].

In addition, a significant association of a SNP in *NAV3* (neuron navigator 3) with chronic postoperative pain was noted in the replication cohort, which was part of the SNPs of interest in the discovery cohort. *NAV3* is a gene predominantly expressed within the central and peripheral nervous systems, is involved in axonal growth and is upregulated 24 h after brain injury [44]. Another SNP in *NAV3,* found to be upregulated in degenerating Alzheimer's disease [45], has been associated with brain development and neuronal differentiation [46]. Interestingly, the *NAV3* gene has been associated with complex regional pain syndrome [47]. However, the direction of the effect of *NAV3* differed between the discovery (protective effect) and replication cohort (risk effect) which could be due to several reasons. One of these could be that the effect of SNPs is subtype specific as already shown in migraine [18]. The replication cohort consisted of a variety of surgeries, while the discovery cohort was focused on hysterectomy, although the hysterectomy approach did differ slightly between hospitals. Furthermore, the limited sample size could skew results to one side and large-scale follow-up and meta-analyses should further verify the direction and the size of the effect.

Genetic studies and GWAS in particular, face several difficulties in the field of chronic postoperative pain. The first challenge is unifying the phenotype and the subphenotypes of chronic postoperative pain [3]. Several efforts have been

Table 2 Association results of the top genomic loci with suggestive genome-wide association in the discovery cohort ($p < 1 \times 10^{-5}$).

SNP and gene information						Discovery cohort			Replication cohort			Meta-analysis	
Index SNP	Variant	Chromosome	Genea	A1	A2	MAF	p value	β (SE)	MAF	p value	β (SE)	p value	β
rs62281806	Intronic	3	FNDC3B	C	T	0.14	5.59E-07	−9326.96 (5606.4)	0.16	0.89	−0.10 (0.71)	0.89	−0.0996
rs10459710	Intronic	15	LOC101927025	C	T	0.19	1.36E-06	1.41 (0.30)	0.08	0.64	−0.43 (1.00)	0.41	0.7358
rs80120866	Intronic	8	LOC100616530	G	T	0.06	2.03E-06	−1846.47 (966.32)	0.05	0.98	−0.04 (121.52)	0.45	−670.3951
rs118184265	Intronic	12	NAV3	A	G	0.05	2.31E-06	−355.21 (153.04)	0.05	0.009	260.97 (124.38)	0.41	−143.5717
rs75361675	Intronic	12	TLL2	C	A	0.10	2.41E-06	−415.93 (287.73)	0.13	0.31	0.70 (0.68)	0.55	−108.2577
rs1514185	Intronic	1	LOC101926964	C	T	0.32	4.21E-06	1.66 (0.43)	0.32	0.77	0.18 (0.61)	0.18	0.988
rs7894047	Intronic	10	RSU1	A	T	0.02	5.33E-06	−10953.7 (58553.4)	0.03	0.07	230.20 (130.02)	0.08	2.302
rs1145324	Intronic	15	IQGAP1	A	G	0.04	6.27E-06	2.53 (0.55)	0.03	0.46	120.98 (15.78)	3.93E-06	2.3863
rs4957810	Intronic	5	PJA2	C	T	0.12	6.69E-06	−419.69 (376.62)	0.17	0.68	0.28 (0.66)	0.74	−40.8342
rs10194315	Intronic	2	LOC105373891	C	T	0.31	6.94E-06	−1.65 (0.43)	0.35	0.99	0.01 (0.48)	0.31	−0.8373
rs117119665	Intronic	15	CRTC3	A	G	0.04	7.00E-06	2.53 (0.55)	0.02	0.31	168.19 (160.38)	2.26E-06	2.4209

A1, major allele; A2, minor allele; MAF, minor allele frequency in the replication cohort, SE, standard error.
[a]Gene annotation on the basis of GRCh37.p13.

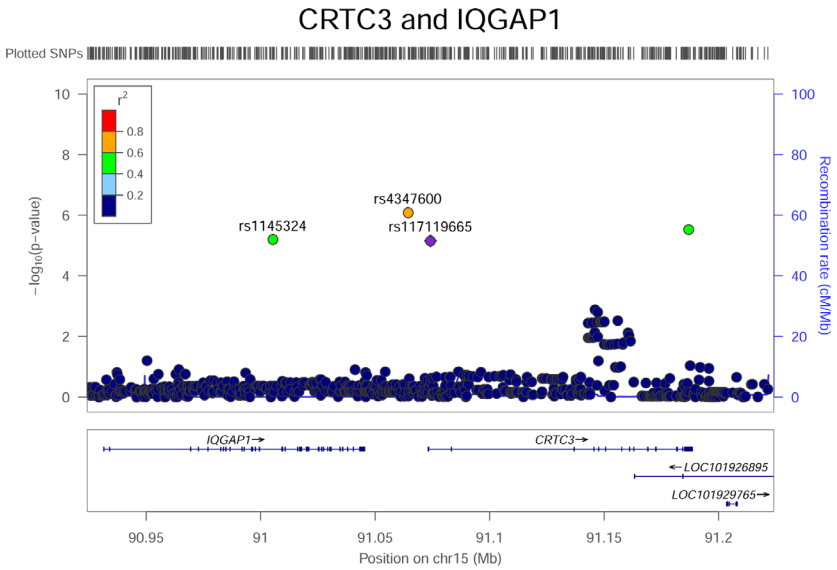

Figure 4 Regional association plot for single-nucleotide polymorphism (SNPs) within *CRTC3* and *IQGAP1* and their association with chronic postoperative pain. The plot shows the chromosomal position of the SNPs (based on 1000 genomes Nov 2014 EUR) in the respective region against the -$\log_{10}$ P values. The SNP with the highest p value in the meta-analysis is represented as a purple diamond. The other SNPs are colour coded according to the extent of linkage disequilibrium with those specific SNPs.

made to improve the phenotyping of chronic postoperative pain [4, 6]. The most recent effort for the new International Classification of Diseases-11 made a further step by specifically defining the sub types of chronic postoperative pain with detailed symptoms per subphenotype [3]. Migraine research has done this subclassification before and a recent genome-wide assocaition meta-analysis identified subtype-specific risk loci, which is a good example of the direction the field should pursue [18]. Combining various indications of chronic postoperative pain helps to identify which SNPs or loci are subtype specific and which are associated with chronic postoperative pain in general. The second challenge is acquiring an adequate sample size, which was rather small in this study. The sample size to find trustworthy results should be multiplied at least a 100-fold, which is not feasible for individual research groups. Instead, following the example of migraine [18], large consortia should be formed to make large sample sizes possible. Combining uniform and detailed (sub-) phenotypes of chronic postoperative pain with a sufficient sample size would make genome-wide associaiton in chronic postoperative pain a success.

Our study provides a foundation for follow-up genome-wide association studies on chronic postoperative pain. Our

analysis only focused on the female sex, but data are available on men and women. Both the detailed information available on both cohorts as well as the availability of both sexes makes this study an ideal starting point for follow-up research. Although none of the SNPs studied reach genome-wide significance, various suggestive signals were identified and replicated in an independent cohort. There are some differences between the discovery cohort and replication cohort, which could influence the results. Firstly, the replication cohort included men and women and approximately 20% received an intervention consisting of methylprednisolone infusion next to the standard postoperative medical regime, whereas the discovery cohort consisted of only women without extra interventions. Women have a higher chance of developing chronic postoperative pain than men [48, 49]. To overcome this difference, we decided to analyse only the women in the cohort who did not undergo methylprednisolone infusion. Secondly, there was a significant 20-year mean age difference between the discovery and the replication cohorts. Younger age is a risk factor for chronic postoperative pain and should be corrected for where possible [48]. To correct for age, a random-effects instead of a fixed effects meta-analysis was conducted as the study design and influential covariates differed between studies.

Currently, a significant portion of the variance in the risk of developing chronic postoperative pain remains to be elucidated, despite the already comprehensively studied psychological, clinical and baseline characteristic risk factors [1, 17, 23]. The identification of genetic risk factors will be a key step towards identifying people at risk of chronic postoperative pain and devising new treatment strategies based on optimised prediction modelling. Although this genome-wide association study alone did not yield genome-wide significant SNPs, the meta-analysis indicated two risk loci for the development of chronic postoperative pain and the replication study provided additional evidence for two loci. Future studies in larger cohorts of patients with chronic postoperative pain will help to elucidate the underlying genetics. Expanding to other surgery types may uncover susceptibility factors for chronic postoperative pain independent of the surgery intervention and possibly subtype-specific loci. Finally, characterisation of the pathways related to chronic postoperative pain has the potential for developing therapeutic approaches to prevent it.

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Appendix 1

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Appendix 2

COMT, catechol-O-methyltransferase; GCH1, GTP cyclohydrolase 1; OPRM1, opioid receptor mu 1; EDNRA, endothelin receptor type A; NAV3, neuron navigator 3; TLL2, tolloid-like protein 2; RSU1, ras suppressor protein 1; TMEM63B, transmembrane protein 63B; PJA2, praja ring finger ubiquitin ligase 2; DLG2, discs large MAGUK scaffold protein 2; FNDC3B, fibronectin type-3 domain containing 3B; KCNS1, potassium voltage-gated channel modifier subfamily S member 1

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Heterozygosity plot showing the heterozygosity rate plotted against the number of missing SNPs per individual.

Table S1. Logistic regression analysis results for the replication cohort extreme case analysis (NRS > 3) versus no pain group (NRS = 0).

Table S2. Logistic regression analysis results for the replication cohort with all patients with pain included (NRS > 0) versus no pain group (NRS = 0).

Table S3. Random effects meta-analysis results of discovery and replication cohort.