Published in final edited form as:

Pain. 2019 March ; 160(3): 561–568. doi:10.1097/j.pain.0000000000001432.

CACNG2 polymorphisms associate with chronic pain following mastectomy

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Abstract

Chronic postmastectomy pain (PMP) imposes a major burden on the quality of life of the ever-increasing number of long-term survivors of breast cancer. An earlier report by Nissenbaum *et al.* (2010) claimed that particular polymorphisms in the gene *CACNG2* are associated with the risk of developing chronic PMP after breast surgery. This information is important as in principle it can inform the surgical, radiological and chemotherapeutic decision-making process in ways that could mitigate the increased risk of chronic pain. In the present study we revisited this claim by independently evaluating the proposed marker haplotype using two different patient cohorts recruited in different research settings. Meta-analysis of these new postmastectomy cohorts and the original cohort confirmed significant association of the *CACNG2* haplotype with PMP. In addition, we tested whether the same markers would predict chronic postsurgical pain in men who underwent surgery for inguinal hernia repair, and whether there is significant genetic association with cutaneous thermal sensitivity in postmastectomy and postherniotomy patients. We found that the biomarker is selective as it did not predict pain following laparoscopic hernia repair and was not associated with pain sensitivity to experimentally applied noxious thermal stimuli. We conclude that the A-C-C haplotype at the three single nucleotide polymorphisms (rs4820242, rs2284015 and rs2284017) in the *CACNG2* gene is associated with increased risk of developing

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We dedicate this paper in remembrance of Prof. Ariel Darvasi (1962-2018), a brilliant geneticist from the Hebrew University of Jerusalem, Israel, and senior author of the initial findings on *CACNG2* effects on pain.

Dr. Belfer contributed to this article in her personal capacity. The views expressed are her own and do not necessarily represent the views of the National Institutes of Health or the United States Government.

PMP. This information may advance current knowledge on pathophysiology of PMP and serve as a step forward prediction of clinical outcomes and personalized pain management.

Summary

Genotyping for three *CACNG2* gene polymorphisms provides important information about the risk of mastectomy pain persistence and serves as a step forward personalized pain management.

Keywords

breast cancer; *CACNG2*; neuropathic pain; pain genetics; mastectomy pain; postsurgical pain

INTRODUCTION

Breast cancer (BRCA) is a common malignancy in women with a lifetime risk exceeding 10%. Due to advances in early radiological and genetic screening and improved treatment protocols, an increasing fraction of patients survive BRCA and go on to live disease-free or in remission for decades. These positive developments, however, have brought to the fore an adverse side effect that compromises the quality of life (QoL) of many BRCA survivors: chronic post-mastectomy pain (PMP). PMP develops in 13.5- 25% of women who have undergone complete or partial surgical removal of the breast with or without cosmetic reconstruction [27]. PMP results from damage to nerves in the axilla and/or chest wall during the initial excision surgery and is exacerbated by a follow-up chemo- and/or radiation therapy [2, 20] . Like other pain conditions with predominant neuropathic component, PMP combines ongoing pain, usually described as “burning” and/or “shooting”, with tactile allodynia (pain upon light touch) [20, 38, 42]. Although not life-threatening, PMP significantly impairs QoL, often for decades after surgery [25]. Available analgesic agents tend to deliver only partial relief in a fraction of women treated, and with significant adverse effects [19].

In 2010, Nissenbaum *et al.* [32] reported that the presence of specific alleles of single-nucleotide polymorphisms (SNPs) and a 3-SNP haplotype (A-C-C) in the human gene *CACNG2* (encoding a voltage-dependent calcium channel gamma subunit 2, also known as the “stargazer gene” in mice) increases the odds of developing chronic PMP. That project began with a whole genome search carried out in a rodent model of neuropathic pain that revealed the mouse homolog *Cacng2* to be a gene of major effect for the pain phenotype. Subsequent genotyping of a cohort of 549 women after mastectomy revealed that the odds of developing PMP was 65% higher in patients carrying two copies of the A-C-C haplotype (Odds Ratio, OR=1.65; p=0.001). We undertook replication of this finding in two independent samples of women who had undergone mastectomy and either developed or did not develop PMP. In addition, we evaluated sex-specificity and generalizability of the association of *CACNG2* alleles to postoperative pain by analyzing a cohort of men who had undergone surgical inguinal hernia repair and either developed or did not develop postherniotomy pain (PHP). Finally, in addition to examining genetic association with clinical pain, we evaluated *CACNG2* effects on sensitivity to experimental pain caused by noxious thermal stimuli in postmastectomy and postherniotomy patients.

METHODS

Information on patient's phenotype and genotype was obtained from three independent "parent projects" that had other objectives. We evaluated the allelic composition of three informative *CACNG2* SNPs from the Nissenbaum *et al.* study (rs4820242, rs2284015 and rs2284017). Detailed information on pain phenotype in the focus pain disorder, as well as on pain sensitivity to experimental stimuli applied at specified locations on the body, had been collected. From the phenotypic data available we selected a set of traits that, in combination, justified assignment of most subjects with PMP or PHP into the dichotomous categories "pain" or "no pain". Criteria for assignment of pain/no pain differed between the cohorts depending on the phenotype data available. In each case, outcome evaluation was done with neither the subject nor the tester being aware of the subject's genotype. Details of the makeup of the cohorts, phenotyping and genotyping procedures were as follows.

University of Pittsburgh Postmastectomy Cohort (UP-PMP)

Subjects: were 482 Caucasian women recruited from a registry of BRCA patients who underwent unilateral (93%) or bilateral (7%) complete mastectomy (33%) or partial mastectomy (67%) at the University of Pittsburgh Medical Center between 1992 and 2010. Mean age was 61 (standard deviation 12) years. All patients gave informed consent to participate in the study. The University of Pittsburgh Institutional Ethics Review Board approved the study protocol. For full details of patient characteristics, see Belfer *et al.* [7].

Phenotyping: The Brief Pain Inventory (BPI) [12] was administered by telephone on average 38 months after surgery. The subjects were also asked to evaluate spontaneous and evoked pain felt in the mastectomy area during the previous week using a 0-10 numeric rating scale (NRS), where 0 denotes "no pain" and 10 denotes "maximum imaginable pain". Following Nissenbaum *et al.* [32] patients who reported no pain (i.e. NRS 0) were assigned to the "pain-free" category. Patients who reported pain severity 1 or greater were assigned to the PMP category. In addition to clinical outcomes, 200 study participants underwent laboratory-based quantitative sensory testing (QST) to evaluate heat pain threshold, heat pain tolerance threshold and response in the cold pressor test (CPT). For heat pain assessment, a contact thermode ($2.56cm^2$, Medoc Advanced Medical Systems, Ramat Yishai, Israel) was applied to 4 pre-marked spots separated by 4 cm proximal to distal along the inner side of the forearm contralateral to the mastectomy (or contralateral to the most painful side in case of bilateral mastectomy). In each trial, thermode temperature increased from a baseline of 32 °C at a rate of 0.5 degrees per second. The subject clicked a mouse to indicate when the stimulus first felt painful, and the thermode temperature was recorded as the "heat pain threshold". This triggered immediate return of the thermode temperature to baseline. A maximal cut-off temperature of 50°C was enforced to avoid skin injury. Next, testing was repeated at the mirror opposite locations. This time the subject was asked to click the mouse when she could no longer tolerate the heat pain ("heat pain tolerance threshold"). Values from four trials of each type were averaged.

Response to noxious cold was assessed by immersion of the right hand up to the wrist in a basin containing 2.5 L of water and ice. Bath temperature remained constant at 3°C over the

course of the test. Time until the subject reported pain in the hand ("cold pain threshold", CPT) and until the pain became intolerable and the hand was withdrawn ("cold pain tolerance threshold") were noted. Immediately after hand withdrawal the subject rated the cold pain intensity on a 0-10 NRS.

Genotyping: DNA was extracted using whole blood samples collected from 21% (99/482) of the study subjects and excised breast tissue samples collected from the other 79% (383/482). Genotyping of the 3 *CACNG2* SNPs was performed using the Taqman platform specifically for these analyses. Genotyping call rates were 99.4% for rs2284015, 99.6% for rs2284017 and 96.9% for rs4820242. All 482 patients had at least one SNP successfully genotyped, and because the haplotype estimation procedure used in these analyses effectively handles missing genotype data, all patients were retained for analyses.

University of Helsinki Postmastectomy Cohort (UH-PMP)

Subjects: The Helsinki PMP cohort consisted of 1,000 women who were to undergo surgery for breast cancer at the Breast Surgery Unit, Helsinki University Central Hospital, Helsinki, Finland between August 2006 and December 2010. Exclusion criteria were bilateral or metastasized cancer, immediate or delayed breast reconstruction, neoadjuvant treatment and age over 75 years. The study protocol has earlier been described in detail by Kaunisto et al. [22].

Phenotyping: The study protocol was approved by the Coordinating Ethics Committee and the Ethics Committee of the Department of Surgery of the Hospital District of Helsinki and Uusimaa. A written informed consent was obtained from each patient. After consent, demographic data and medical history were collected.

Before surgery, cold pain tolerance was assessed. In the cold pain tolerance test, the patient immersed her hand into cold (2-4 °C) water (Julabo USA Inc, Allenton, PA). Pain intensity was recorded every 15s using NRS. The patients were advised to withdraw the hand from the water at any time when the pain felt intolerable, the maximum time was 90 seconds. The time the patients kept the hand in the water was recorded and pain intensity was assessed with NRS at withdrawal. Cold pain data were available from 900 patients due to the unavailability of the test device at the beginning of the study. Surgery was either breast-conserving surgery or mastectomy with axillary surgery. A questionnaire was sent to the patients 12 months after surgery. Pain in the operative area during previous week was assessed with numerical rating scale (NRS 0-10, zero representing "no pain" and 10 "worst pain imaginable"). Pain with NRS ≥1 in any area (breast, axilla, arm) was used for statistical analysis [27].

DNA extraction and genotyping:

DNA was extracted from peripheral blood using the Autopure LS automated DNA purification instrument (Gentra Systems Inc, Minneapolis, MN). All genotyping was done blindly to phenotype information. The GWA genotype data were produced at the Wellcome Trust Sanger Institute (Hinxton, UK) on the Human OmniExpress Illumina BeadChip (Illumina, Inc., San Diego, CA, USA). After stringent sample quality control procedures,

SNPs were filtered based on minor allele frequency (MAF>0.005), Hardy-Weinberg equilibrium (HWE p>1×10-6) and success rate (>0.97). The final cohort consisted of 926 individual DNAs with a mean genotyping success rate of 0.997. The data were then imputed using SHAPEIT v2 and IMPUTE2 against the 1000 Genomes Phase 3 reference panel.

Postherniotomy cohort (PHP)

Subjects: were n=198 Caucasian men (average age 55 (standard deviation 13) years) who underwent unilateral elective laparoscopic trans-abdominal groin hernia repair at the Centre for Minimal Invasive Surgery, Bethesda Krankenhaus Stuttgart, Stuttgart, Germany [1]. Candidates were excluded if they were unable to understand instructions, had body mass index >35 kg/m^2, any current malignant disease, abuse of alcohol, diseases impairing central or peripheral nervous system function, other abdominal hernias, previous operations that might have affected sensitivity in the inguinal region, or advanced dementia. All participants gave informed consent. The local ethics committee of the Centre for Minimal Invasive Surgery approved the study protocol.

Phenotyping: Six months after surgery a detailed clinical examination including quantitative sensory testing was performed to assess sensory function and clinical pain. Pain was assessed using the NRS (similar to the PMP cohorts), and patients were dichotomized into “pain” and “no pain” groups using the same criterion as for the PMP cohort.

Heat pain sensitivity in groin and arm was assessed using the Modular Sensory Analyzer (Somedic AB, Hörby, Sweden) following a protocol described in detail elsewhere [6]. Briefly, four stimuli for each temperature of 45°, 46°, 47°, and 48°C (16 heat stimulations in total) were administered in the groin area in a semi-randomized order keeping the patient unaware of the ensuing temperature in relation to the previous, throughout the study. The ramp rate was 5°C/s with a return rate of 1°C/s. The temperature was maintained for 5 s with a 30-s inter-stimulus period. At the end of each stimulation, the patient reported NRS pain (0-10), and the average pain assessed for each temperature was calculated. For full details of patient characteristics, see Aasvang et al. [1].

Genotyping: Genomic DNA was extracted from whole blood samples using the QIAamp DNA mini kit (Qiagen, Venlo, Netherlands) and following manufacturer instructions. The 3 *CACNG2* SNPs were genotyped using predesigned TaqMan assays (Applied Biosystems, Foster City, CA). Allele-specific fluorescence signals were distinguished by measuring endpoint 6-FAM or VIC fluorescence intensities at 508 nm and 560 nm, respectively. Genotypes were inferred using the Sequence Detection System Software (ver. 1.7, Applied Biosystems). Genotyping error rate was determined by re-genotyping 25% of the samples, randomly chosen, for each locus. Repeated sample concordance was > 99.5% and genotype call rate was ≥98%.

Statistical analyses

Genotypes were tested for Hardy-Weinberg equilibrium using Fisher exact probabilities tests and the 3 SNPs of interest were found to be in equilibrium in all of the cohorts studied. In each cohort we evaluated the association between *CACNG2* haplotypes and the presence or

absence of criterion clinical pain using the *haplo.score* and *haplo.glm* functions from the *haplo.stats* R package [24]. These two functions were specifically developed to test for association of a trait with haplotypes, when linkage phase is unknown. *Haplo.score* allows for testing the effects of individual haplotypes and the global haplotype effect at a specific locus. We used p-values produced by this function to test for general and individual haplotype effects on each trait in the cohorts. *Haplo.glm* is used to estimate the effect of each haplotype (odds ratio for binary traits and regression coefficients for continuous traits), with 95% confidence intervals. Of note, the default baseline (reference) haplotype in *haplo.glm* is the most frequent haplotype in the population. Because our haplotype of interest (A-C-C) was also the most frequent haplotype in both cohorts, as an alternative we chose to use G-G-T, the haplotype consisting of alleles complimentary to A-C-C, as our background haplotype to estimate the effects in regression.

Association of individual SNP genotypes with binary outcomes was tested using a logistic regression. An additive (or multiplicative) genetic model was assumed throughout the analyses. *A priori* we were primarily interested in corroborating the individual SNPs rs4820242, rs2284015 and rs2284017, and the core haplotype A-C-C of these SNPs, which were identified as significantly associated with PMP in the Nissenbaum et al. [32] study. These genotypes were tested first, without adjustment of p-values for multiple testing. If the association of the A-C-C haplotype was not significant (i.e. $p > 0.05$), we went on to evaluate the “global score statistic”, i.e. we performed a simultaneous test for all haplotypes in this locus. If the global score statistic was significant ($p < 0.05$), we then evaluated individually the significance of each of its component haplotypes after applying Bonferroni correction. Haplotypes with estimated population frequencies <1% were grouped into one category (all participants were Caucasians). For completeness, if the global score statistic was not significant, hap-scores and associated p-values of the individual haplotypes were nevertheless reported. Associations of *CACNG2* haplotypes with the continuous QST variables (pain threshold and pain tolerance to experimentally applied heat and cold stimuli) were evaluated using the *haplo.glm* function with the identity link; associations of individual SNP alleles were evaluated using linear regression. Association results from individual cohorts were combined in a meta-analysis using the inverse variance method as implemented in MetaXL software [17].

RESULTS

University of Pittsburgh Postmastectomy Cohort (UP-PMP)

CACNG2 polymorphisms were genotyped at SNPs rs4820242, rs2284015 and rs2284017 in 482 women postmastectomy in whom PMP status was determined using our pre-defined criteria. PMP was present in 45% (216/482) of the study participants, a frequency similar to the 39% (215/549) reported by Nissenbaum *et al*. SNPs rs4820242, rs2284015, and rs2284017 had minor allele frequency of 0.41, 0.30, and 0.48 respectively (Table 1), similar to the ones reported by Nissenbaum et al. (0.35, 0.22, and 0.40, respectively), and were in Hardy-Weinberg equilibrium ($p > 0.15$). None of the individual SNPs was significantly associated with PMP (Table 2). The A-C-C haplotype (allele frequency = 0.33) was significantly associated with PMP (p-value= 0.033, odds of PMP increasing 1.40 times per 1

haplotype copy, Table 2). This haplotype appeared at elevated frequency in women who had PMP versus those without PMP (A-C-C haplotype frequency 0.35 vs. 0.29, respectively). There was no difference if the mastectomy was complete or local (lumpectomy). The results replicated the specific associations between PMP and *CACNG2* polymorphisms originally reported by Nissenbaum et al. in an independent cohort of postmastectomy patients.

Women in the PMP cohort were also evaluated for sensitivity to experimental noxious thermal stimuli. There was no statistically significant association of *CACNG2* SNPs or haplotypes with the cold pressure pain (after correction for multiple testing).

University of Helsinki Postmastectomy Cohort (UH-PMP)

CACNG2 polymorphisms were directly genotyped at rs4820242 and imputed at rs2284015 and rs2284017 (*info*-statistic 0.9965 and 0.9959, respectively). A total of 800 subjects had complete phenotypic and genotypic data needed for the analyses. PMP (NRS>3) was present in 14% (110/800) and PMP (NRS ≥1) in 62% (495/800) of the study participants. SNPs rs4820242, rs2284015, and rs2284017 had minor allele frequency of 44%, 40%, and 40% respectively (Table 1) and were in Hardy-Weinberg equilibrium ($p > 0.05$). None of the individual SNPs was significantly associated with PMP (Table 2). The A-C-C haplotype (allele frequency = 0.25) was not significantly associated with PMP (p-value= 0.76). Also, there was no statistically significant association of *CACNG2* SNPs or haplotypes with the cold pressure pain.

Postherniotomy cohort (PHP)

Genotype data were obtained for 187 male patients who underwent laparoscopic hernia repair (for 11 patients, there was not sufficient quantity or quality of DNA sample). Criterion chronic PHP pain was present in 31.6% (59/187). Of the 3 target SNPs (minor allele frequency 0.34, 0.22, and 0.41 for rs4820242, rs2284015 and rs2284017, respectively), only the rs2284015 was significantly ($p = 0.024$) associated with pain. Interestingly, this SNP decreased the risk of pain in PHP (OR 0.55 per copy of C allele). A-C-C haplotype (allele frequency 0.40) was not significantly associated with PHP (OR = 0.97, $p = 0.90$, Table 2).

For quantitative sensory testing, C-allele at rs2284017 was associated with increased heat pain threshold in the groin ($p=0.018$) and approached significance in association with heat pain threshold in arm ($p=0.075$). A-C-C haplotype approached a nominal significance threshold 0.05 in association with heat pain threshold in groin ($p = 0.052$). None of the QST associations remained significant following correction for multiple testing (Table 3).

Meta-analysis of *CACNG2* haplotype effects

We performed meta-analysis of *CACNG2* haplotype associations with chronic PMP using results from Nissenbaum et al., and our findings from two post-mastectomy cohorts. Results are shown in Fig. 1 (panel A). A-C-C haplotype effects on chronic postmastectomy pain in UP-PMP cohort and Nissenbaum et al. study are virtually identical. The overall combined odds ratio is 1.26 (95%CI 1.07 to 1.47) and the p-value is 0.005.

DISCUSSION

Nissenbaum et al. (2010) reported a significant association between chronic PMP and three *CACNG2* SNPs (and haplotype A-C-C) in a cohort of women who underwent mastectomy. This gene was chosen as a sole candidate based on results of a whole-genome search in mice with contrasting pain phenotype after nerve injury. The potential value of *CACNG2* as a risk factor for developing PMP motivated us to undertake a replication study in independent patient samples collected in different countries by different investigators using equivalent, but not identical, criteria for defining PMP. The main result of our study was a successful replication of the original findings for the A-C-C haplotype in a postmastectomy cohort and in a meta-analysis of all three PMP cohorts. The *CACNG2* A-C-C haplotype is therefore a likely risk factor for chronic chest-wall pain following surgical mastectomy.

Generality

To investigate the generality of the association, we evaluated the association of the *CACNG2* SNPs and A-C-C haplotype with clinical pain in a cohort of patients who underwent surgical herniotomy. In addition, we evaluated the association with sensitivity to pain in response to experimentally applied noxious stimuli in both cohorts (PMP and PHP). Only one SNP (rs2284015) was significantly associated with PHP, and its effect was in a direction opposite to one found by Nissenbaum et al. in PMP patients [32] . This is likely due to differences in the underlying pain mechanism. The mechanism of PHP is uncertain, but it is unlikely to be neuropathic. Compared to thoracic surgeries, incisions in the abdominal region (e.g. appendectomy, caesarian section) do not tend to induce chronic pain and the transient incisional pain that does occur is not typically neuropathic in quality [23]. PHP is more likely to be nociceptive and/or inflammatory in origin due, for example, to intra-abdominal tissue adhesions. We are not aware of adequately powered studies that tested whether *CACNG2* might be linked to any other type of painful neuropathy. However, association with pain caused by cutaneous inflammation has been noted [39, 40] .

Our observations add to the growing appreciation that pain related to different biological mechanisms may have different genetic underpinnings. The same applies to experimental pain. Studies in animals and in humans suggest that high vs. low sensitivity to acutely applied thermal stimuli, for example, does not necessarily correlate genetically with pain evoked by mechanical or irritant chemical stimuli, or with models of clinical pain [3, 16, 29, 30]. A few of the associations of experimental pain endpoints in PMP and PHP had p-values <0.05 (Table 3). None of these associations remained significant after correction for the total number of tests performed.

The observed *CACNG2* effects on postmastectomy but not postherniotomy chronic pain can also be also attributed to sex specificity that was reported to many genes controlling pain across species [5]. In general, there are three major scenarios of sex-specific differences in the effect on of a gene on a trait or behavior: sex-specific effects (for example, GTP cyclohydrolase (*GCH1*) haplotype affects susceptibility to pain crises only in women [9]), sex-biased effects (for example, catechol-O-methyltransferase (*COMT*) haplotype affects pain in both sexes but to different degrees [8]), and sex-antagonistic effects (for example, SNP in Mu-opioid receptor gene, *OPRM1*, affects postsurgical low back pain in both sexes

but in opposite directions [33]). Therefore, it is possible that *CACNG2* haplotype contributes to chronic pain conditions only in women. Future studies in women with persistent pain of non-neuropathic origin such as fibromyalgia may distinguish between mechanism- and sex-specific effects of *CACNG2*.

Mechanism

The association of *CACNG2* with PMP can provide insight into the pathophysiology of the pain condition and ultimately into the mechanism whereby the genetic polymorphisms biases the system towards increased pain. *CACNG2* encodes a transmembrane protein (stargazin) that is a subunit of neuronal voltage-sensitive Ca^{2+} channels $Ca_v2.1$ and $Ca_v2.2$, important determinants of neuronal excitability [11, 18, 21, 37]. Other Ca^{2+} channel subunits also play a role in pain. Mutations in *CACNA1A*, for example, are a known cause of familial hemiplegic migraine [14] and *CACNA2δ* is the main binding site of gabapentin and pregabalin, widely prescribed analgesics for neuropathic pain [41]. Stargazin is also a transmembrane AMPA-R regulatory protein (TARP), involved in both the trafficking of AMPA-type glutamate receptors to the neuronal membrane and synaptic cleft [4, 13, 28, 35] and in the modulation of AMPA-R kinetics [10, 35]. Any of these biological mechanisms could be relevant to PMP. Interestingly, *CACNG2* mutations are also strongly linked to epilepsy [26, 31, 34, 36], a condition that, like neuropathic pain, is fundamentally an outcome of neuronal hyperexcitability [15]. Indeed, the leading drugs used in the medical treatment of neuropathic pain conditions, including PMP, are anti-epileptics. One of the three informative *CACNG2* SNPs is located near exon 3 (rs4820242) and the other two are near exon 4 in an independent haplotype block. This suggests the possibility of two independent effects on the gene product. Further research is required to identify the specific causal chain that links the A-C-C haplotype with increased risk of PMP. Identifying the detailed mechanism(s) has the potential to advance our understanding of pain physiology.

Study limitations

All three PMP cohorts used here for meta-analysis were comprised of self-reported white subjects. Although no ancestry informative markers (AIMs) were genotyped to control for genetic admixture or population stratification of the study samples, risk of confounding by population stratification and admixture in the final meta-analysis is deemed minimal. It is very unlikely that the direction of population stratification effects, if any, is the same in all three PMP cohorts.

Use of analgesics can be an important factor affecting pain self-report in postoperative populations and the corresponding genetic associations. However, in the UP-PMP cohort *CACNG2* haplotypes were not associated (p-value = 0.58) with the reported use of analgesics for PMP. Therefore, it is unlikely that analgesics significantly influence the reported genetic associations in our study. This study was sufficiently powered to evaluate the association of *CACNG2* haplotypes with the occurrence of any present pain (NRS > 0) due to large proportion of such cases in the cohort. Moderate or severe pain (NRS > 3) is important from a clinical perspective; however, due to smaller proportion of subjects with moderate or severe pain, larger cohorts will be needed for genetic association analyses and prognostic evaluation of biomarkers. In further studies, the prognostic role of *CACNG2*

markers should be tested in larger cohorts across different races and ethnicities. Also, in this study, we did not evaluate the interaction between *CACNG2* haplotype and other risk factors (e.g., clinical, psychosocial and demographic) identified for PMP [7] and PHP [1]. Additional information on the link between *CACNG2* polymorphisms and pain following mastectomy could advance understanding of the pathophysiology and mechanisms underlying PMP. It could also lead to the use of *CACNG2* genotyping as a means of predicting clinical outcomes and hence serve to guide personalized pain management and perhaps personalized pain prevention.

ACKNOWLEDGEMENTS

We gratefully acknowledge all the patients who agreed to take part in the study, without whose participation and cooperation this work would not have been possible. Supported by The University of Pittsburgh School of Medicine internal funds (to IB), the Seymour and Cecile Alpert Chair in Pain Research (to MD). The authors seen and approved the final version of this manuscript. We declare no conflicts of interest with respect to the manuscript.

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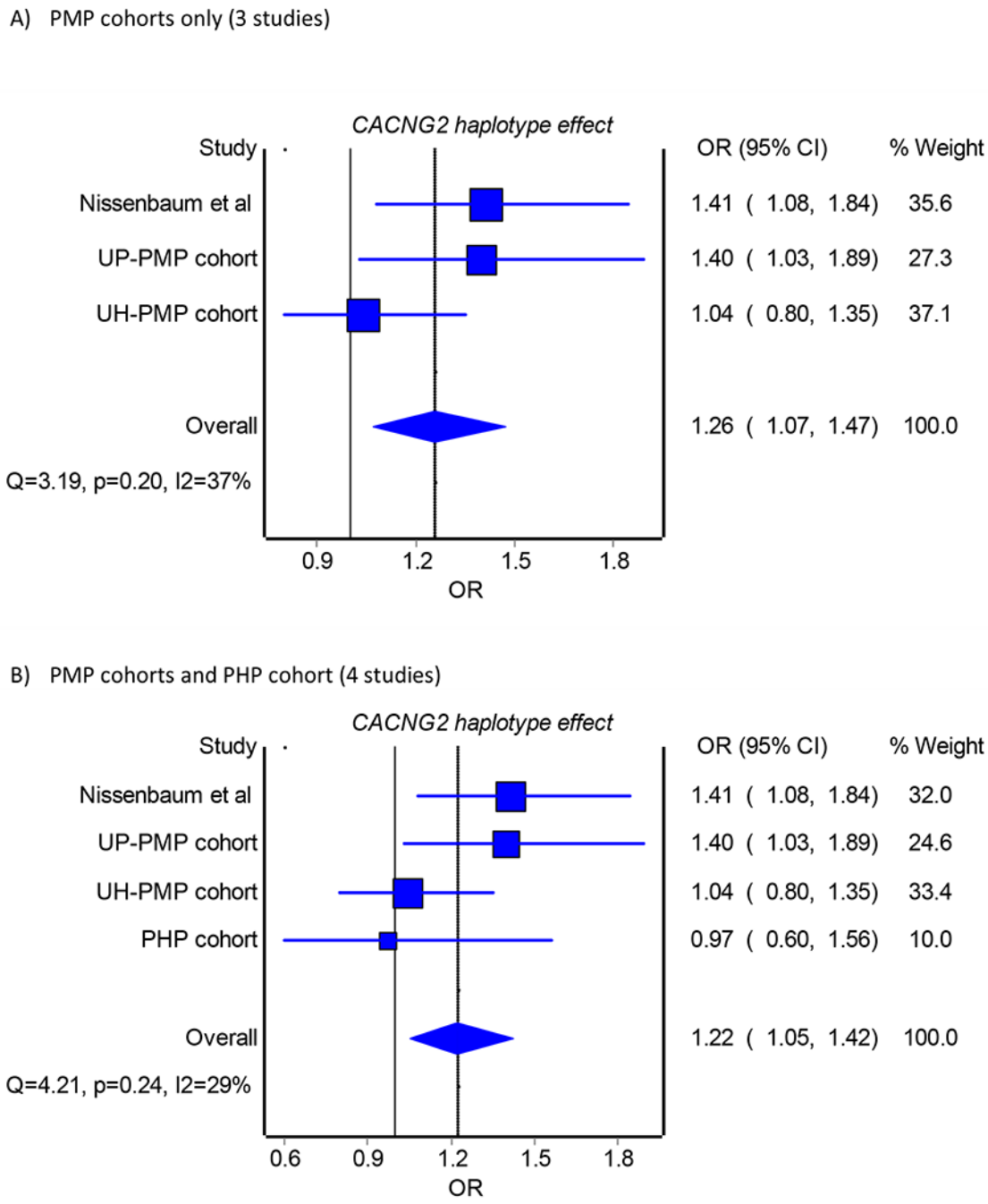

Figure 1.

Meta-analysis of A-C-C effects on chronic pain in four cohorts. A) Overall p-value for the combined odds ratio for three PMP cohorts is 0.005. B) P-value for the combined odds ratio for PMP and PHP cohorts is 0.0087. % weight is based on the inverse variance of the estimated effect (width of the confidence interval).

Table 1.

Demographic characteristics and allele frequencies in postmastectomy pain (PMP) and postherniotomy pain (PHP) cohorts

	PMP(Pittsburg)	PMP(Helsinki)	PHP
Females, n	482	800	0
Males, n	0	0	187
Age, yrs (std dev)	61(12)	57(9)	55(13)
Haplotype	*allele freq*	*allele freq*	*allele freq*
ACC	0.34	0.25	0.40
ACT	0.10	0.14	0.10
AGT	0.18	0.17	0.16
GCC	0.21	0.15	0.19
GCT	0.07	0.06	0.08
GGT	0.11	0.23	0.07
SNP			
rs4820242 (A>G)	0.41	0.44	0.34
rs2284015 (C>G)	0.30	0.40	0.22
rs2284017 (C>T)	0.48	0.60	0.41

Table 2.

The *CACNG2* A-C-C haplotype significantly associates with PMP in the University of Pittsburg postmastectomy cohort

Cohort	n	Sex	Outcome	Allele	OR (95% CI)[a]	OR P-value
UP-PMP	482	Females	Pain at 6 mo	A-C-C	1.40 (1.03, 1.89)	**0.033**
				rs4820242 (A)	1.16 (0.90, 1.49)	0.24
				rs2284015 (C)	1.04 (0.79, 1.39)	0.77
				rs2284017 (C)	1.28 (0.99, 1.67)	0.062
UH-PMP	800	Females	Pain at 12 mo	A-C-C	1.04 (0.80, 1.35)	0.76
				rs4820242 (A)	0.90 (0.73, 1.11)	0.33
				rs2284015 (C)	0.87 (0.70, 1.08)	0.21
				rs2284017 (C)	0.97 (0.79, 1.21)	0.81
PHP	187	Males	Pain at 6 mo	A-C-C	0.97 (0.60, 1.56)	0.90
				rs4820242 (A)	1.27 (0.78, 2.04)	0.34
				rs2284015 (C)	0.55 (0.33, 0.93)	0.024 **
				rs2284017 (C)	0.72 (0.47, 1.12)	0.15

[a]Odds ratio for PMP, per one copy of the allele; for A-C-C haplotype effect, all other haplotypes were pooled into one reference ("baseline") group.

**Having the C-allele at this locus reduces the odds of having PHP.

Table 3.

Association of *CACNG2* SNPs and haplotypes with experimental measures of pain sensitivity

Cohort	n	Gender	Outcome	Genetic variant	Effect (95% CI)[a]	Effect P-value**
UP-PMP	200	Females	Cold pressor tolerance	A-C-C	−8.1 (−16.3, 0.1)	0.055
				rs4820242 (A)	−0.05 (−0.58, 0.48)	0.85
				rs2284015 (C)	−7.7 (−15.2, −0.1)	0.049
				rs2284017 (C)	−9.8 (−17.1, −2.6)	0.009
UP-PMP	200	Females	Heat tolerance	A-C-C	−0.03 (−0.60, 0.54)	0.91
				rs4820242 (A)	−2.9 (−19.6, 13.9)	0.74
				rs2284015 (C)	7.5 (−10.4, 25.4)	0.41
				rs2284017 (C)	−1.6 (−19.0, 15.7)	0.86
UP-PMP	200	Females	Heat threshold	A-C-C	−0.21 (−0.93, 0.51)	0.56
				rs4820242 (A)	−0.32 (−0.92, 0.28)	0.29
				rs2284015 (C)	0.23 (−0.42, 0.88)	0.50
				rs2284017 (C)	0.00 (−0.63, 0.62)	0.99
UH-PMP	830	Females	Cold pain tolerance	A-C-C	1.12 (−2.65, 4.90)	0.56
				rs4820242 (A)	0.60 (−2.47, 3.67)	0.95
				rs2284015 (C)	−3.40 (−6.48, −0.32)	0.031
				rs2284017 (C)	−1.49 (−4.58, 1.60)	0.24
PHP	187	Males	Heat pain threshold (groin)	A-C-C	0.55(0.0, 1.10)	0.052
				rs4820242 (A)	0.13 (−0.44, 0.69)	0.65
				rs2284015 (C)	0.30 (−0.32, 0.92)	0.34
				rs2284017 (C)	0.62 (0.11, 1.13)	0.018
PHP	187	Males	Heat pain threshold (arm)	A-C-C	0.46 (−0.24, 1.16)	0.20
				rs4820242 (A)	0.18 (−0.52, 0.87)	0.62
				rs2284015 (C)	0.49 (−0.27, 1.24)	0.21
				rs2284017 (C)	0.58 (−0.055, 1.21)	0.075

[a]Effect using the additive genetic model (i.e. phenotype mean difference per one copy of the allele); for A-C-C haplotype effect, all other haplotypes were pooled into one reference (“baseline”) group.

**No p-values remained significant after applying the corrected significance threshold (corrected alpha for 20 tests is 0.0025).