


ORIGINAL ARTICLE

Genetic polymorphisms and prediction of chronic post-surgical pain after hysterectomy—a subgroup analysis of a multicenter cohort study

Daisy M. N. Hoofwijk^{1,2}  | Roel R. I. van Reij^{1,2} | Bart P. F. Rutten^{2,3} | Gunter Kenis^{2,3} | Maurice Theunissen^{1,2} | Elbert A. Joosten^{1,2} | Wolfgang F. Buhre^{1,2} | Nynke J. van den Hoogen^{1,2}

¹Department of Anesthesiology and Pain Medicine, Maastricht University Medical Center+, Maastricht, The Netherlands

²School for Mental Health and Neuroscience (MHeNs), Faculty of Health, Medicine and Life Sciences, Maastricht University Medical Center+, Maastricht, The Netherlands

³Faculty of Health, Medicine and Life Sciences, Department of Psychiatry and Neuropsychology, Maastricht University Medical Center+, Maastricht, The Netherlands

Correspondence

Daisy M. N. Hoofwijk, Department of Anesthesiology and Pain Medicine, Maastricht University Medical Center+, PO Box 5800, 6202 AZ Maastricht, The Netherlands.
Email: d.hoofwijk@gmail.com

Background: Chronic post-surgical pain (CPSP) is a serious problem. Clinical and psychological variables have not been able to explain all observed variance in prevalence and severity of CPSP. The first objective is to determine the association between genetic polymorphisms and the prevalence of CPSP after hysterectomy. The second objective is to analyze if the implementation of genetic polymorphisms into a previously performed clinical and psychological predictor analysis on the development of CPSP after hysterectomy will improve its discriminatory power.

Methods: A prospective multicenter cohort study was performed in patients undergoing hysterectomy for benign indication. Clinical and psychological variables were collected by questionnaires in the week before surgery, post-operatively up to day 4, 3 and 12 months after hysterectomy. Blood was collected and 16 polymorphisms previously suggested to be correlated to CPSP (COMT, GCH1, KCNS1, CACNG2, and OPRM1) were genotyped. Logistic regression analyses were performed.

Results: Three hundred and forty-five patients were available for the genetic analyses. The prevalence of CPSP 3 months post-operatively was 10.5% and after 12 months 7.9%. The polymorphism rs4818 within the COMT gene was associated with the prevalence of CPSP after 3 months. No polymorphisms were associated with CPSP after 12 months. The addition of rs4818 to the prediction model did not change its discriminatory power significantly.

Conclusion: The rs4818 polymorphism within the COMT gene was associated with the prevalence of CPSP 3 months after hysterectomy, but the implementation of rs4818 into the prediction model did not significantly improve the chance of identifying hysterectomy patients at risk for CPSP.

Trial registry number: Number NTR2702, Dutch Trial Register (<http://www.trialregister.nl/trialreg/index.asp>).

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2019 The Authors. Acta Anaesthesiologica Scandinavica published by John Wiley & Sons Ltd on behalf of Acta Anaesthesiologica Scandinavica Foundation.

1 | INTRODUCTION

Chronic post-surgical pain (CPSP) is a common problem affecting up to 60% of surgical patients and is associated with negative outcomes such as a reduced quality of life and socioeconomic consequences.¹⁻⁶ The prevalence of CPSP after hysterectomy is estimated between 5% and 32%, but prevalences up to 50% have been reported.^{7,8} However, definitions for CPSP are highly variable between studies, and therefore reported prevalences can vary widely as well.

Demographic, clinical, and psychological risk factors for the development of CPSP have been well documented and include young age, procedure-related risk factors, preexisting pain, acute post-operative pain, pain catastrophizing, surgical fear, and lack of optimism.^{1,2,9-11} Unfortunately, these factors have not been able to explain all the observed variance in the prevalence and severity of CPSP. For this reason, the study and implementation of these polymorphisms have become of interest to further improve prediction models for CPSP. Data from twin studies and human pedigrees have estimated that the heritability of chronic pain generally ranges from 30% to 70%.¹² Especially polymorphisms in genes that encode for proteins involved in nerve conduction and transmission, opioid signaling, and inflammatory processes have been suggested to play an important role in the development of CPSP.¹³⁻¹⁶ Identifying these genes could play an important role in finding targets for treatment.

Multiple studies have been performed that describe the association between genetic polymorphisms and the prevalence or severity of CPSP.¹³⁻¹⁹ Based on a systematic review, an association between the prevalence or severity of CPSP and single-nucleotide polymorphisms (SNPs) within the COMT (catechol-O-methyl transferase) gene, opioid receptor genes, potassium channel genes, GCH1 (GTP cyclohydrolase 1) gene, CACNG2 (calcium channel voltage gate-dependent gamma subunit 2) gene, CHRNA6 (cholinergic receptor nicotinic alpha 6) gene, P2X7R (P2X purinoceptor 7) gene, cytokine-associated genes, human leukocyte antigens, DRD2 (dopamine receptor D2) gene, and ATXN1 (ataxin 1) gene was suggested.²⁰ Nevertheless, most of these polymorphisms were researched by only one study, with the exception of polymorphisms within the COMT, OPRM1, GCH1, and KCNS1 genes.^{18,21-24} Furthermore, this systematic review revealed that several studies were of retrospective design, and most studies lacked statistical power and had only performed exploratory statistical analyses without correcting for multiple testing or known confounders.²⁰ Interestingly, none of the studies used in the systematic review incorporated genetic polymorphisms in pre-existing prediction models for CPSP (ie, based on demographic, clinical, and psychological factors). As a result, it is unknown whether the inclusion of associated genetic polymorphisms will improve the discriminatory power of the existing clinical and psychological prediction models for the development of CPSP. It needs no further explanation that the latter is of significant clinical importance.

This study is a subgroup analysis of a large multicenter cohort study that has been published elsewhere.²⁵ The current subgroup

Editorial comments

Persisting pain after surgery has gained attention as a major pain problem. Several surgical and post-operative factors have been shown to influence its development, but the link between genetics and persistent/chronic post-surgical pain has not been completely elucidated. In this hysterectomy study, two polymorphisms within the COMT gene were found to be associated with post-operative pain at 3 months after hysterectomy.

analysis has two objectives. The first objective is to determine if genetic polymorphisms in the COMT, OPRM1, GCH1, CACNG2, and KCNS1 genes are associated with the prevalence of CPSP after hysterectomy. The second objective is to determine if the implementation of these genetic polymorphisms into a previously performed clinical and psychological predictor analysis for CPSP in this population will be able to improve its discriminatory power.²⁵

2 | METHODS

2.1 | Study population

The study was registered at the Dutch Trial Register under the number NTR2702 (<http://www.trialregister.nl/trialreg/index.asp>). An elaborate description of the study protocol has been published elsewhere.²⁵ In short, a multicenter prospective cohort study was performed. Patients planned to undergo hysterectomy for a benign indication were included in one of four hospitals in the Netherlands: Maastricht University Medical Center+ (MUMC+) Maastricht, the Catharina Hospital (CzE) Eindhoven, the Máxima Medical Center (MMC) Veldhoven, and the Orbis Medical Center (OMC) Sittard-Geleen. Hysterectomy patients were chosen because they present a homogenous patient group, and this procedure was performed frequently in the participating hospitals. Inclusion took place between September 2010 and January 2014. Inclusion criteria were age between 18 and 65 years, good command of the Dutch language, elective surgery, and total or subtotal hysterectomy with or without oophorectomy, and all types of surgical approach. Exclusion criteria were history of cancer, illiteracy, and cognitive impairment. Patients who reported a malignancy or underwent another surgical procedure during the first post-operative year were also excluded. Approval of the local Medical Ethical Committee was obtained in all hospitals and all patients gave informed consent before participation.

2.2 | Collection of pre-operative variables and predictors

Patients were asked to fill out a baseline questionnaire before undergoing the hysterectomy. This questionnaire contained questions about demographics, general health status and comorbidity,

pre-operative pain, and several psychological predictors. Pre-operative pain was measured by using an adapted version of the Brief Pain Inventory, which determines the severity, location, duration, and intensity of pain during the last week.²⁶ An 11-item Numeric Rating Scale (NRS; where 0 = no pain, and 10 = worst pain imaginable) was used to measure pain intensity. Patients were asked to rate pre-operative pain related to the indication for the planned hysterectomy (eg, lower abdominal pain) and pain not related to the indication for the planned hysterectomy separately. The Douleur Neuropathique 4 (DN4) was used to assess the neuropathic character of the pain. This instrument originally consists of 10 items, but only the 7 self-reported items were used (DN4-interview).^{25,27} In agreement with previous literature, a score of ≥ 3 was defined as neuropathic pain.²⁷

Several psychological predictors were included. Surgical fear was measured by the 8-item surgical fear questionnaire, of which four items measure short-term aspects of surgical fear and four items measure long-term aspects of fear.²⁸ The 13-item Pain Catastrophizing Scale (PCS) was used to measure pain catastrophizing.²⁹ Optimism was assessed using the 10-item revised Life Orientation Test (LOT-R).³⁰ Social support was measured by the 19-item Medical Outcomes Study–Social Support Survey (MOS-SSS), and by asking the patient for the number of close friends and relatives available for support.³¹ Depression was assessed with the Center for Epidemiological Studies–Depression (CES-D) questionnaire.³² Psychological well-being was assessed by the 12-item Well-Being Questionnaire (W-BQ12), which measures positive well-being, energy, and negative well-being.³³ Finally, also expected pain at the fourth post-operative day, and childhood abuse were assessed.

2.3 | Collection of perioperative variables and predictors

Surgery-related and anesthesia-related variables were collected at the time of the procedure. These included type of incision, total or subtotal hysterectomy, with or without oophorectomy, indication for the hysterectomy, type of anesthesia, post-operative analgesic use, duration of the surgery, amount of blood loss, complications, and the amount of years of experience of the attending gynecologist.

Acute post-operative pain was assessed each day during the first four post-operative days. Patients were asked to rate their average and highest pain at rest and during movement, as well as the use of any analgesics. At the fourth post-operative day, the DN4-interview was used to assess neuropathic pain.

2.4 | Collection of outcomes measures

Questionnaires were used to measure pain severity at 3 and 12 months after hysterectomy. Patients were asked to rate hysterectomy-related and non-hysterectomy-related pain separately. Predictor analysis was performed with the highest hysterectomy-related pain during the last week. In accordance with previous research, an NRS > 3 was defined as moderate to severe pain.^{2,25,34,35}

2.5 | Genotyping and quality control

Peripheral blood samples were obtained from the included patients. DNA was extracted from these samples at the Department of Clinical Genetics of the Maastricht University Medical Center+ (Maastricht, the Netherlands). Genotyping of SNPs within the following genes was performed at the Institute of Human Genetics at the UniKlinikum (Bonn, Germany) using the Infinium PsychArray v1.2 BeadChip (Illumina Inc., San Diego, CA, USA) and a customized Iplex array: COMT (rs4633, rs6269, rs4818, rs4680), GCH1 (rs10483639, rs3783641, rs8007267), KCNS1 (rs13043825, rs734784), CACNG2 (rs2284015, rs2284017, rs2284018, rs4820242, rs1883988), and OPRM1 (563649, rs1799971). These genes and polymorphisms were selected because they have previously been reported to be linked to CPSP.²⁰

Genotypes were called using BeadStudio (Illumina Inc.). Quality control parameters included SNP callrate $< 95\%$, subject callrate $< 95\%$, deviation of the Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$) and removal of rare variants with a minor allele frequency < 0.01 . After basic quality control, heterozygosity was tested and outliers (> 3 standard deviations) were removed. Relatedness between the subjects was tested and one member of each pair was removed at random if relatedness was detected. All SNPs with A \leftrightarrow T or C \leftrightarrow G polymorphisms were removed from the set. After these control steps, the SNPs were pruned to remove SNPs in LD ($r^2 > 0.02$). Principal components analysis was performed and the first two principal components were analyzed. The ancestry of the subjects was determined using HapMap, which showed that the outliers in the principal component analysis had a background other than Caucasian and were therefore removed from the sample.

Genotype imputation was performed using the imputation stepwise approach implemented in Minimac3³⁶ and Eagle v2.3³⁷. HRC r1.1 2016 panel was the reference panel, with population set to European.³⁸ The output of the imputation was checked with the Will Rayner tool. Afterward additional quality control was performed with genotype probability > 0.9 , quality threshold was set at 0.5, INFO-score > 0.4 and minor allele frequency and genotyping rate was checked again.

2.6 | Statistical analysis

Patient characteristics and mean pain scores were evaluated using descriptive statistics. Before performing any other analyses, missing data were imputed by predictive mean matching. This method imputes values within the range of observed values in the data set, and is therefore robust to non-normal distribution of variables. The number of imputations was set to 5. The results demonstrated are the pooled results based on these five data sets. In order to reduce the number of psychological predictors, a factor analysis with oblimin rotation was performed. Principal component analyses revealed two factors, which were named surgery-related worries (based on the SFQ and PCS) and general psychological robustness (based on the LOT-R, CES-D, and W-BQ12).²⁵

The association between the genetic polymorphisms and CPSP at 3 and 12 months was tested by univariate logistic regression analyses. An additive genetic model was tested, in which the number of minor alleles (0, 1, or 2) was defined as the candidate predictor. A P value of <0.05 was considered statistically significant.

In order to evaluate the effect of addition of genetic polymorphisms to a previously performed predictor analysis based on clinical and psychological variables, analyses were repeated using the same data.²⁵ Separate analyses were performed for CPSP after 3 and 12 months. We first performed univariate logistic regression analyses on the same candidate predictors as were used in our previous publication (ie, participating hospital, age, type of anesthesia, type of incision, paid job, educational level, American Society of Anesthesiology (ASA) physical status, number of pregnancies, surgery-related worries, general psychological robustness, history of sexual abuse, hysterectomy-related baseline pain, non-hysterectomy-related baseline pain, neuropathic pain at post-operative day 4, training of attending gynecologist, surgery-related infection during the first three post-operative months). For clinical and psychological predictors, the variable was only included in the multivariate logistic regression analysis if the univariate P value was <0.1 . Before including any of the genetic predictors in the regression model, correction for multiple testing was performed. This was done according to the method described by Galwey et al., in which the effective number of tests is used instead of the actual number of tests.^{39,40} In our study the effective number of tests was 11, which means that the SNP was only included in the regression model if the P value in the univariate analysis was <0.0091 ($0.1/11$). After inclusion of all predictor variables in the regression model, a backward elimination analysis was performed until all remaining P values were <0.5 for clinical and psychological variables or <0.0045 ($0.05/11$) for genetic variables.

For the variables hospital, age, type of anesthesia, and type of incision, a forced entry method was used in our previous publication, but because they were all nonsignificant, we did not use a forced entry in this analysis. After the final clinical and psychological prediction model was determined, the area under the curve (AUC) was calculated to investigate the model's discriminatory accuracy. After that, the genetic polymorphisms that were statistically significant in univariate logistic regression analyses were added to the multivariate prediction model, after which a backward elimination analysis was performed until all P values were <0.05 . The AUC was calculated for this final prediction model, and allows for determination whether the use of genetic analysis in clinical prediction models improves the chance of identifying patients at risk for CPSP after hysterectomy.

3 | RESULTS

3.1 | Flowchart and patient characteristics

Figure 1 demonstrates the flowchart of inclusion and follow-up, which is an extension of the flowchart of our previous publication.²⁵ Of the 517 patients that provided informed consent, 428 patients (82.8%) provided baseline data and returned at least one

of the follow-up questionnaires. Of these patients, 3 patients were excluded because no blood was available for genotyping and 80 patients were excluded because of insufficient quality of the isolated DNA, leaving 345 patients (80.6%) with useful data for the genetic analyses. Of these 345 patients, 332 patients (96.2%) returned the 3-month follow-up questionnaire, and 305 patients (91.9%) returned the 12-month follow-up questionnaire.

Baseline patient characteristics are displayed in Table 1 and perioperative data are shown in Table 2. All patients included were females of European descent. The average age at the time of hysterectomy was 46.9 years (SD 7.2). The most frequently performed procedure was vaginal hysterectomy (65.8%), and the most frequently performed anesthetic technique was general anesthesia (88.4%). Most patients were ASA physical class I (54.8%) or II (40.3%). The prevalence of moderate to severe pre-operative pain (NRS > 3) was rather high; 49.3% reported pain related to the indication for the planned hysterectomy and 34.2% reported pain not related to the planned hysterectomy. Pain at the fourth post-operative day was also common; 37.5% of patients scored an NRS >3 , and 20.8% of all patients scored ≥ 3 at the DN4-interview.

3.2 | Genetic polymorphisms and CPSP

Three months after hysterectomy, 35 of 332 patients (10.5%) experienced moderate to severe hysterectomy-related pain (NRS > 3). Twelve months after hysterectomy, the prevalence decreased to 24 of 305 patients (7.9%). A more elaborate description of the different aspects of the reported pain (eg, location, onset, intermittence, pain interference, etc.) and analgesic treatment is presented elsewhere.²⁵

Table 3 shows the location, minor alleles, and minor allelic frequencies of all included SNPs. Almost all SNPs followed Hardy-Weinberg equilibrium, with the exception of rs2284018. This is probably caused by the fact that the genotype TT was not present in our population. Therefore, the results of polymorphism rs2284018 need to be interpreted with caution. Table 4 demonstrates the results of the univariate regression analyses without correction for multiple testing. CPSP 3 months after hysterectomy was statistically significantly associated with the minor allele of rs4818 (OR [95%CI] 1.99 [1.19-3.33], P value 0.009), and rs6269 (1.97 [1.18-3.28], P value 0.01) within the COMT gene. After correction for multiple testing, only rs4818 was eligible for inclusion in the prediction model for CPSP. All other tested SNPs, within the GCH1, CACNG, KCNS1, and OPRM1 genes, were not significantly associated with the presence of moderate to severe hysterectomy-related pain 3 months after the surgery. No association was found between the included SNPs and the severity of pain 12 months after hysterectomy.

3.3 | Discriminatory power of prediction models

Because no SNPs were significantly associated with CPSP 12 months after hysterectomy in the univariate logistic regression analyses, we will only describe the prediction model for CPSP 3 months after hysterectomy. The following demographic, clinical, and psychosocial

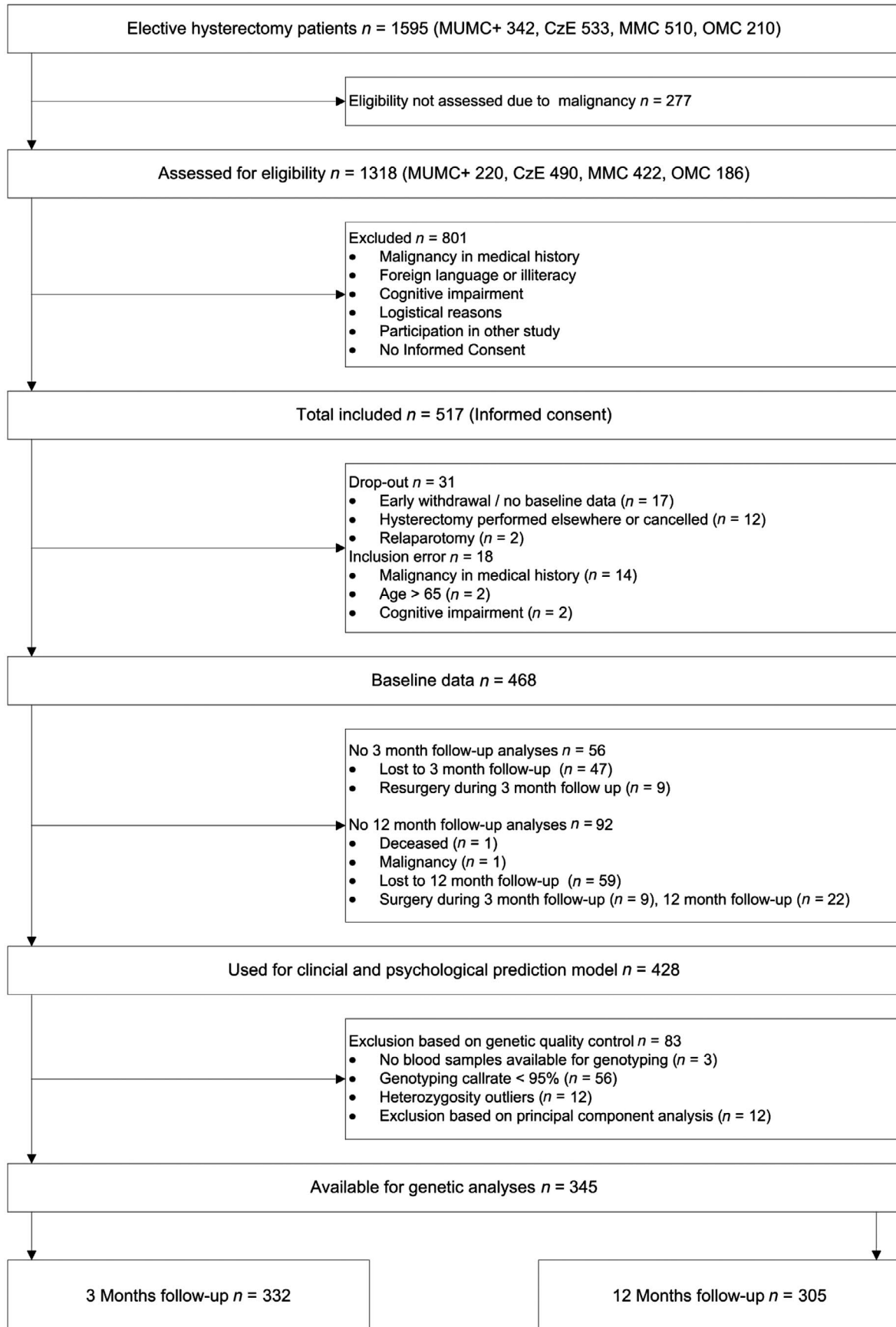


FIGURE 1 Flowchart of patient inclusion

TABLE 1 Baseline patient characteristics

	Baseline sample (n = 345)
Center	
Maastricht UMC+	116 (33.6%)
CzE	106 (30.7%)
MMC	87 (25.2%)
OMC	36 (10.4%)
Age (mean, SD)	46.9 (7.2)
Employment	
Paid job	252 (73.3%)
No paid job	92 (26.7%)
Educational level	
No/lower	51 (14.8%)
Intermediate	214 (62.2%)
University/higher	79 (23.0%)
ASA physical class	
I	189 (54.8%)
II	139 (40.3%)
III	5 (1.4%)
Pre-operative pain	
Hysterectomy-related pain (highest NRS > 3)	170 (49.3%)
Non-hysterectomy-related pain (highest NRS > 3)	118 (34.2%)
History of sexual abuse	
No	272 (79.5%)
Yes	70 (20.5%)
Number of pregnancies (mean, SD)	2.5 (1.2)
Indication for hysterectomy (more than one possible)	
Leiomyoma	105 (30.4%)
Prolapse	64 (18.6%)
Menorrhagia/metrorrhagia	170 (49.3%)
Dysmenorrhoea	22 (6.4%)
Endometriosis/adenomyosis	18 (5.2%)
Abdominal pain	18 (5.2%)
Cervical dysplasia	20 (5.8%)
Other	19 (5.5%)

Abbreviations: ASA, American Society of Anesthesiologist; Maastricht UMC+, Maastricht University Medical Center+; CzE, Catharina Hospital Eindhoven; MMC, Máxima Medical Center Veldhoven; OMC, Orbis Medical Center Sittard-Geleen; NRS, Numeric Rating Scale; SD, Standard Deviation.

variables were not associated with CPSP at 3 months in the univariate analyses and were therefore not used in the clinical prediction model: participating hospital, type of anesthesia, type of incision, educational level, ASA physical status, general psychological robustness, history of sexual abuse, and training of attending gynecologist.

The results of the logistic regression analysis for CPSP after 3 months are shown in Table 5. After performing the backward

TABLE 2 Perioperative data

	Baseline sample (n = 345)
Anesthesia type	
General	296 (85.8%)
Spinal	40 (11.6%)
General & epidural	9 (2.6%)
Hysterectomy procedure	
Total hysterectomy	328 (95.1%)
Subtotal hysterectomy	14 (4.1%)
Oophorectomy	
No	317 (93%)
Unilateral oophorectomy	10 (2.9%)
Bilateral oophorectomy	14 (4.1%)
Incision type	
Total laparoscopic	65 (18.8%)
Vaginal- or laparoscopic-assisted vaginal	227 (65.8%)
Abdominal incision	53 (15.4%)
Years of training of gynecologist (mean, SD)	14.2 (9.9)
Pain at rest at PACU (1 hour after hysterectomy)	
NRS 0-3	168 (54.5%)
NRS 4-10	140 (45.5%)
Pain at post-operative day 4 (highest NRS last 24 hours)	
NRS 0-3	188 (62.5%)
NRS 4-10	113 (37.5%)
DN4 at day 4	
0-2	228 (79.2%)
≥3	60 (20.8%)

Abbreviations: PACU, Post-Anesthesia Care Unit; NRS, Numeric Rating Scale; SD, Standard Deviation; DN4, Douleur Neuropathique 4.

elimination analysis, the following variables were included in the clinical prediction model: employment status, pre-operative hysterectomy-related pain, neuropathic pain at the fourth post-operative day, and self-reported infection during the first 3 post-operative months. The area under the receiver operating characteristic curve (AUC) for this clinical model was 0.78.

In step 2, COMT rs4818 was added to the model. Rs4818 remained significant in the model, with an odds ratio of 2.50 (1.36-4.60). The addition of this variable increased the AUC of the prediction model from 0.78 (95% CI 0.69-0.86) to 0.82 (0.74-0.91), but this was not statistically significant.

4 | DISCUSSION

The first objective of this study was to determine if genetic polymorphisms in the COMT, OPRM1, GCH1, CACNG2, and KCNS1 genes are associated with the prevalence of CPSP after

TABLE 3 Description of included polymorphisms within our patient population

Gene	SNP	Chromosome	Major/minor allele	MAF	HWE P*
COMT	rs4633	22	T/C	0.44	0.01
	rs6269	22	A/G	0.36	0.01
	rs4818	22	C/G	0.36	0.04
	rs4680	22	A/G	0.44	0.01
GCH1	rs10483639	14	G/C	0.18	0.49
	rs3783641	14	T/A	0.18	0.02
	rs8007267	14	C/T	0.17	0.01
KCNS1	rs13043825	20	C/T	0.28	0.21
	rs734784	20	T/C	0.45	0.58
CACNG	rs2284015	22	C/G	0.27	0.76
	rs2284017	22	C/T	0.47	0.32
	rs2284018	22	C/T	0.22	<0.001
	rs4820242	22	A/G	0.37	0.48
	rs1883988	22	G/A	0.28	0.95
OPRM1	rs563649	6	C/T	0.10	0.52
	rs1799971	6	A/G	0.14	0.06

Abbreviations: SNP, Single-Nucleotide Polymorphism; MAF, Minor Allelic Frequency; HWE, Hardy-Weinberg equilibrium.

*P value HWE: cut-off point of 0.003 (0.05/16).

TABLE 4 Association between included polymorphisms and chronic post-surgical pain (CPSP) at 3 and 12 months after hysterectomy

Gene	SNP	CPSP at 3 months (n = 332)		CPSP at 12 months (n = 305)	
		OR (95% CI)	P	OR (95% CI)	P
COMT	rs4633	1.59 (0.95-2.66)	0.08	1.09 (0.59-2.02)	0.78
	rs6269	1.97 (1.19-3.26)	0.01	1.22 (0.67-2.24)	0.52
	rs4818	1.99 (1.19-3.33)	0.009	1.35 (0.73-2.48)	0.34
	rs4680	1.58 (0.94-2.65)	0.08	1.20 (0.65-2.22)	0.57
GCH1	rs10483639	1.11 (0.61-2.03)	0.74	1.11 (0.54-2.31)	0.77
	rs3783641	1.04 (0.57-1.90)	0.90	1.13 (0.56-2.30)	0.73
	rs8007267	1.34 (0.76-2.36)	0.32	1.08 (0.52-2.26)	0.84
KCNS1	rs13043825	0.86 (0.48-1.53)	0.61	0.91 (0.46-1.77)	0.77
	rs734784	0.77 (0.47-1.29)	0.32	0.85 (0.47-1.55)	0.60
CACNG	rs2284015	0.86 (0.49-1.14)	0.61	0.80 (0.40-1.58)	0.51
	rs2284017	1.15 (0.90-1.47)	0.57	0.89 (0.50-1.60)	0.70
	rs2284018	0.75 (0.36-1.56)	0.45	0.77 (0.32-1.84)	0.56
	rs4820242	1.35 (0.81-2.26)	0.25	1.19 (0.65-2.18)	0.57
	rs1883988	0.83 (0.47-1.47)	0.53	0.75 (0.38-1.49)	0.41
OPRM1	rs563649	1.40 (0.63-3.12)	0.41	0.37 (0.09-1.62)	0.19
	rs1799971	1.25 (0.60-2.60)	0.56	0.54 (0.18-1.62)	0.27

Abbreviations: SNP, Single-Nucleotide Polymorphism; OR, Odds ratio. Bold indicates significant P values.

hysterectomy. This study shows that the rs4818 polymorphism within the COMT gene is associated with the presence of moderate to severe chronic post-surgical pain, 3 months after hysterectomy. The COMT gene encodes for the enzyme catechol-O-methyl transferase, which is essential in the breakdown of catecholamines. Polymorphisms within this gene can cause altered COMT enzyme

activity.^{41,42} The COMT gene has been studied extensively in relation to several pain syndromes. Associations have been found for experimental pain, for nonsurgical chronic pain, for acute post-operative pain and for CPSP.⁴³⁻⁴⁸ In line with our findings, Rut et al. reported a relationship between increasing severity of CPSP after lumbar discectomy and the minor allele (G) of rs4818, the

TABLE 5 Prediction model for CPSP 3 months after hysterectomy, based on demographic, clinical, psychological, and genetic factors

Independent variable	OR (95% CI)	P value	AUC
Step 1			0.78
Age			
Per year	NS	N/A	
Employment			
Paid job vs no paid job	0.39 (0.17-0.91)	0.03	
Pregnancies			
Per number	NS	N/A	
Surgery-related worries			
Per point (range -1.68-2.60)	NS	N/A	
Pre-operative hysterectomy-related pain			
Per point on NRS (range 0-10)	1.18 (1.04-1.35)	0.01	
Pre-operative non-hysterectomy-related pain			
Per point on NRS (range 0-10)	NS	N/A	
DN4-interview at post-operative day 4			
≥3 vs 0-2	4.65 (1.77-12.18)	0.002	
Self-reported infection first 3 post-operative months			
Yes vs no	6.81 (2.66-17.45)	<0.001	
Step 2			0.82
rs4818 (COMT gene)			
Per number of minor alleles	2.50 (1.36-4.60)	0.003	

Abbreviation: NRS, Numeric Rating Scale; DN4, Douleur Neuropathique 4; OR, Odds Ratio; CI, Confidence Interval; AUC, Area Under the Curve; NS, Not significant.

minor allele (G) of rs4680, and homozygosity for the minor allele (T) of rs4633.¹⁸ On the other hand, in contrast to our findings, Rut and colleagues also described an association between less severe CPSP and the minor allele (G) of rs6269. Patients with the minor allele of rs6269 have been reported to experience more severe pre-operative pain in their study.¹⁸ Two studies that investigated pain in the acute post-operative phase, demonstrated a relationship between increased pain severity and the presence of one or two minor alleles of rs4818 in patients after third molar extraction or surgery for breast cancer.^{23,45} An association between pain sensitivity in an experimental setting and several polymorphisms within the COMT gene in women with temporomandibular joint disorder has been reported, as patients with the major allele (A) of and the major allele (C) of rs4818 are more sensitive to pain.⁴³ An association between several COMT polymorphisms and pain-related functional impairment 6 months after inguinal hernia surgery

has been described, as the presence of one or more minor alleles of rs4633 and rs6269 were associated with a protective effect.⁴⁹

In sum, many studies reported associations between pain and polymorphisms within the COMT gene. However, the allele responsible for an increase in pain sensitivity or severity is not identical in all studies. Polymorphisms associated with increased pain severity in some studies were associated with a protective effect in other publications.^{18,23,43,45,48} Differences in types of pain (eg, post-operative pain vs experimental pain and functional impairment) might be a possible explanation for these variations. Future studies are needed to further analyze the relation between types of pain, pain sensitivity or severity, and polymorphisms in the COMT gene. Obviously, it is very important to know the allele responsible for an increase in pain severity before these COMT polymorphisms can be included in general pain prediction models.

In this study, we were unable to demonstrate an association between the prevalence of CPSP after hysterectomy and polymorphisms within the GCH1, KCNS1, CACNG, and OPRM1 genes. Associations between CPSP and these polymorphisms have been found in previous publications.^{13,15,16,19,20,24} It is very likely that our present study lacks statistical power to find an association between these polymorphisms and the presence of CPSP 3 and 12 months after hysterectomy. Future studies, in larger patient cohorts, are necessary to determine whether an association between these genes and CPSP exists.

The second objective of our study was to determine if the addition of genetic polymorphisms to the pre-existing clinical and psychological prediction model for CPSP after hysterectomy would be able to improve the discriminatory power of the model. Our study demonstrates, for the first time, that the addition of polymorphisms within the COMT gene to the pre-existing prediction model improved the discriminatory power of the model from "fair" (AUC 0.7-0.8) to "good" (AUC 0.8-0.9). Even though the difference in AUC between a clinical prediction model and a prediction model with additional genetic factors was not statistically significant, we did show a trend toward improvement, and even small improvements in prediction value can be of clinical relevance in personalizing risk profiles and tailoring preventative strategies.⁴⁹ Although many studies have found associations between polymorphisms in several genes and the prevalence and severity of CPSP,²⁰ until now the additional value of using genetic polymorphisms in predicting CPSP has not been described. The additional value of using polymorphisms in pain-related functional impairment after inguinal hernia repair has been reported, but pain-related functional impairment is a different outcome than CPSP.⁴⁹ Nevertheless, comparable findings between the two studies suggest that COMT polymorphisms play an important role in mediating pain processes during the chronic post-operative period.^{20,49} Future studies will be needed to determine whether polymorphisms within other genes might be able to even further improve clinical and psychological prediction models.

Many studies have shown that psychological predictors, such as pain catastrophizing, surgical fear, and lack of optimism are associated with the prevalence and severity of CPSP, but we were not able

to confirm these findings in the present study.^{2,9,50} For this study, the considerable number of psychological predictors was reduced to two variables (ie, general psychological robustness and surgery-related worries) based on a factor analysis.²⁵ Combining multiple psychological predictors into a single variable might explain why we did not find a significant association between psychological robustness and CPSP. On the other hand, lack of statistical power might also be a reason for the absence of an association between psychological predictors and CPSP. However, there was to be enough power to detect an association between genetic polymorphisms and CPSP. It is worth mentioning that we did find a statistically significant association between CPSP and surgery-related worries in our previous publication. Differences in statistically significant risk factors between our original and the current risk model were also observed for employment status and pain not related to the planned hysterectomy. Several explanations are available for these differences in risk models. First of all, a different statistical approach was used. In our original study, a mixed model analysis was performed, because this approach was very robust for missing data, but the downside of this approach was the fact that the time point after surgery (ie, 3 or 12 months after surgery) was included as one of the variables. In our current risk model, we preferred to present separate results for 3 and 12 months after surgery in order to be able to present the influence of genetic factors at these different time points. Therefore, a separate logistic regression analysis was performed for both time points; robustness for missing data was assured using multiple imputation. We hypothesize that these differences in statistical approach, in combination with the loss to follow-up of 70 patients (due to insufficient quality of the genetic material) and the resulting loss of statistical power, has led to the mentioned differences in both risk models. We believe that larger patient cohorts will lead to more stable and robust risk models. Future studies are therefore needed to determine the role of simultaneous use of psychological and genetic variables for the prediction of CPSP.

Important strengths of our study are both its prospective longitudinal design, as well as the homogeneity of the patient sample. All patients were of female gender, the same ethnicity, and underwent the same surgery for benign indication. However, our study also has limitations. First of all, the sample size was relatively small for genetic research. The study was powered to perform a regression analysis with clinical and psychological variables. It is likely that larger sample sizes are necessary to detect the association between individual genetic polymorphisms and the prevalence of CPSP. In addition to this, the prevalence of CPSP was relatively low (only 35 patients after 3 months, and 24 patients after 12 months), which further decreases the statistical power. This could explain why we did find an association between polymorphisms and pain 3 months after hysterectomy, but not 12 months. The second limitation is that we only studied polymorphisms that have already been studied by other authors. No genome-wide association studies (GWAS) have been performed yet with regard to CPSP.

Future studies on CPSP and genetics should focus on GWAS. This is the only way to identify all polymorphisms that can possibly

be associated with the development of CPSP. This approach would require larger sample sizes and preferably homogeneous patient populations with a clearly defined pain phenotype. At the moment, our research group is working on a GWAS to determine if other polymorphisms are associated with CPSP after hysterectomy. After promising polymorphisms have been identified by GWAS, these polymorphisms can be tested and validated in large groups of patients, and various surgical procedures. We hypothesize that individual genes explain only a small part of the variation in pain phenotype, whereas a combination of multiple genes of interest might be able to explain a larger portion. This combination of polymorphisms can then be combined with clinical and psychological predictors to compose personalized risk profiles for standard procedures.

In conclusion, our study demonstrates that two polymorphisms within the COMT gene are associated with the prevalence of moderate to severe CPSP 3 months after hysterectomy. In line with recent literature, our results further indicate that polymorphisms within the COMT gene play an important role in post-operative mechanisms of pain processing. Addition of the rs4818 polymorphism to the prediction model showed a trend toward improvement. In order to determine the role of other genes on the development of CPSP, more research in larger patient cohorts is needed.

DECLARATION OF INTERESTS

D.M.N. Hoofwijk: No interests declared.

R.R.I. van Reij: No interests declared.

B.P.F. Rutten: No interests declared.

G. Kenis: No interests declared.

M. Theunissen: No interests declared

E.A. Joosten: No interests declared

W.F.F.A Buhre: Chief investigator PRODIGY Trial funded by Medtronic, Chief investigator Phoenix/Thetys Trial funded by B. Braun.

N.J van den Hoogen: No interests declared.

AUTHORS CONTRIBUTIONS

D.M.N. Hoofwijk was responsible for the genetic analyses, statistical analyses, data interpretation, figure creation, and writing of the paper. R.R.I. van Reij was responsible for the genetic analyses, statistical analyses, data interpretation, figure creation, and writing of the paper. B.P.F. Rutten was responsible for the data interpretation and writing of the paper. G. Kenis was responsible for the data interpretation and writing of the paper. M. Theunissen was responsible for the patient inclusion, data collection, statistical analyses, data interpretation, figure creation, and writing of the paper. E.A. Joosten was responsible for the data interpretation and writing of the paper. W.F.F.A Buhre was responsible for writing of the paper. N.J. van den Hoogen was responsible for the genetic analyses, data interpretation, and writing of the paper.

ORCID

Daisy M. N. Hoofwijk  <https://orcid.org/0000-0002-8860-4516>

REFERENCES

- Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. *Lancet*. 2006;367(9522):1618-1625.
- Hoofwijk DM, Fiddelaers AA, Peters ML, et al. Prevalence and predictive factors of chronic postsurgical pain and poor global recovery one year after outpatient surgery. *Clin J Pain*. 2015;31(12):1017-1025.
- Simanski CJP, Althaus A, Hoederath S, et al. Incidence of chronic postsurgical pain (CPSP) after general surgery. *Pain Med*. 2014;15(7):1222-1229.
- Lavand'homme P. The progression from acute to chronic pain. *Curr Opin Anaesthesiol*. 2011;24(5):545-550.
- Parsons B, Schaefer C, Mann R, et al. Economic and humanistic burden of post-trauma and post-surgical neuropathic pain among adults in the United States. *J Pain Res*. 2013;6:459-469.
- Peng Z, Li H, Zhang C, Qian X, Feng Z, Zhu S. A retrospective study of chronic post-surgical pain following thoracic surgery: prevalence, risk factors, incidence of neuropathic component, and impact on quality of life. *PLoS ONE*. 2014;9(2):e90014.
- Brandsborg B, Nikolajsen L, Kehlet H, Jensen TS. Chronic pain after hysterectomy. *Acta Anaesthesiol Scand*. 2008;52(3):327-331.
- Pinto PR, McIntyre T, Nogueira-Silva C, Almeida A, Araujo-Soares V. Risk factors for persistent postsurgical pain in women undergoing hysterectomy due to benign causes: a prospective predictive study. *J Pain*. 2012;13(11):1045-1057.
- Macrae WA. Chronic post-surgical pain: 10 years on. *Br J Anaesth*. 2008;101(1):77-86.
- Katz J, Seltzer Z. Transition from acute to chronic postsurgical pain: risk factors and protective factors. *Expert Rev Neurother*. 2009;9(5):723-744.
- Peters ML, Sommer M, de Rijke JM, et al. Somatic and psychologic predictors of long-term unfavorable outcome after surgical intervention. *Ann Surg*. 2007;245(3):487-494.
- Clarke H, Katz J, Flor H, Rietschel M, Diehl SR, Seltzer Z. Genetics of chronic post-surgical pain: a crucial step toward personal pain medicine. *Can J Anaesth*. 2015;62(3):294-303.
- Montes A, Roca G, Sabate S, et al. Genetic and clinical factors associated with chronic postsurgical pain after hernia repair, hysterectomy, and thoracotomy: a two-year multicenter cohort study. *Anesthesiology*. 2015;122(5):1123-1141.
- Wieskopf JS, Mathur J, Limapichat W, et al. The nicotinic alpha6 subunit gene determines variability in chronic pain sensitivity via cross-inhibition of P2X2/3 receptors. *Sci Transl Med*. 2015;7(287):287ra72.
- Langford DJ, Paul SM, West CM, et al. Variations in potassium channel genes are associated with distinct trajectories of persistent breast pain after breast cancer surgery. *Pain*. 2015;156(3):371-380.
- Nissenbaum J, Devor M, Seltzer Z, et al. Susceptibility to chronic pain following nerve injury is genetically affected by CACNG2. *Genome Res*. 2010;20(9):1180-1190.
- Stephens K, Cooper BA, West C, et al. Associations between cytokine gene variations and severe persistent breast pain in women following breast cancer surgery. *J Pain*. 2014;15(2):169-180.
- Rut M, Machoy-Mokrzyńska A, Ręclawowicz D, et al. Influence of variation in the catechol-O-methyltransferase gene on the clinical outcome after lumbar spine surgery for one-level symptomatic disc disease: a report on 176 cases. *Acta Neurochir*. 2014;156(2):245-252.
- Kolesnikov Y, Gabovits B, Levin A, et al. Chronic pain after lower abdominal surgery: do catechol-O-methyl transferase/opioid receptor mu-1 polymorphisms contribute? *Mol Pain*. 2013;9:19.
- Hoofwijk DM, van Reij RR, Rutten BP, Kenis G, Buhre WF, Joosten EA. Genetic polymorphisms and their association with the prevalence and severity of chronic postsurgical pain: a systematic review. *Br J Anaesth*. 2016;117(6):708-719.
- George SZ, Wallace MR, Wright TW, et al. Evidence for a biopsychosocial influence on shoulder pain: pain catastrophizing and catechol-O-methyltransferase (COMT) diplotype predict clinical pain ratings. *Pain*. 2008;136(1-2):53-61.
- Kolesnikov Y, Gabovits B, Levin A, Voiko E, Veske A. Combined catechol-O-methyltransferase and mu-opioid receptor gene polymorphisms affect morphine postoperative analgesia and central side effects. *Anesth Analg*. 2011;112(2):448-453.
- Lee PJ, Delaney P, Keogh J, Sleeman D, Shorten GD. Catecholamine-o-methyltransferase polymorphisms are associated with postoperative pain intensity. *Clin J Pain*. 2011;27(2):93-101.
- Costigan M, Belfer I, Griffin RS, et al. Multiple chronic pain states are associated with a common amino acid-changing allele in KCNS1. *Brain: A J Neurol*. 2010;133(9):2519-2527.
- Theunissen M, Peters ML, Schepers J, et al. Recovery 3 and 12 months after hysterectomy: epidemiology and predictors of chronic pain, physical functioning, and global surgical recovery. *Medicine*. 2016;95(26):e3980.
- Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. *Ann Acad Med Singapore*. 1994;23(2):129-138.
- Bouhassira D, Attal N, Alchaar H, et al. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain*. 2005;114(1-2):29-36.
- Theunissen M, Peters ML, Schouten EGW, et al. Validation of the surgical fear questionnaire in adult patients waiting for elective surgery. *PLoS ONE*. 2014;9(6):e100225.
- Sullivan MJL, Bishop SR, Pivik J. The pain catastrophizing scale: development and validation. *Psychol Assess*. 1995;7(4):524-532.
- Scheier MF, Carver CS, Bridges MW. Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. *J Pers Soc Psychol*. 1994;67(6):1063-1078.
- Sherbourne CD, Stewart AL. The MOS social support survey. *Soc Sci Med*. 1991;32(6):705-714.
- Radloff L. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psych Meas*. 1977;1:385-401.
- Bradley C. The 12-item well-being questionnaire: origins, current stage of development, and availability. *Diabetes Care*. 2000;23(6):875.
- Gerbershagen HJ, Rothaug J, Kalkman CJ, Meissner W. Determination of moderate-to-severe postoperative pain on the numeric rating scale: a cut-off point analysis applying four different methods. *Br J Anaesth*. 2011;107(4):619-626.
- Dihle A, Helseth S, Paul SM, Miaskowski C. The exploration of the establishment of cutpoints to categorize the severity of acute postoperative pain. *Clin J Pain*. 2006;22(7):617-624.
- Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and methods. *Nat Genet*. 2016;48(10):1284-1287.
- Loh P-R, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype Reference Consortium panel. *Nat Genet*. 2016;48(11):1443-1448.
- McCarthy S, Das S, Kretschmar W, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet*. 2016;48(10):1279-1283.
- Galwey NW. A new measure of the effective number of tests, a practical tool for comparing families of non-independent significance tests. *Genet Epidemiol*. 2009;33(7):559-568.
- Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity*. 2005;95(3):221-227.
- Diatchenko L, Nackley AG, Slade GD, Fillingim RB, Maixner W. Idiopathic pain disorders—pathways of vulnerability. *Pain*. 2006;123(3):226-230.

42. Nackley AG, Shabalina SA, Tchivileva IE, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science*. 2006;314(5807):1930-1933.
43. Diatchenko L, Slade GD, Nackley AG, et al. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet*. 2005;14(1):135-143.
44. Diatchenko L, Nackley AG, Slade GD, et al. Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. *Pain*. 2006;125(3):216-224.
45. Kambur O, Kaunisto MA, Tikkanen E, Leal SM, Ripatti S, Kalso EA. Effect of catechol-o-methyltransferase-gene (COMT) variants on experimental and acute postoperative pain in 1,000 women undergoing surgery for breast cancer. *Anesthesiology*. 2013;119(6):1422-1433.
46. Candiotti KA, Yang Z, Buric D, et al. Catechol-o-methyltransferase polymorphisms predict opioid consumption in postoperative pain. *Anesth Analg*. 2014;119(5):1194-1200.
47. Tammimaki A, Mannisto PT. Catechol-O-methyltransferase gene polymorphism and chronic human pain: a systematic review and meta-analysis. *Pharmacogenet Genomics*. 2012;22(9):673-691.
48. Belfer I, Segall S. COMT genetic variants and pain. *Drugs of today*. 2011;47(6):457-467.
49. Belfer I, Dai F, Kehlet H, et al. Association of functional variations in COMT and GCH1 genes with postherniotomy pain and related impairment. *Pain*. 2015;156(2):273-279.
50. Theunissen HM, Peters ML, Bruce J, Gramke HF, Marcus MA. Preoperative anxiety and catastrophizing: a systematic review and meta-analysis of the association with chronic postsurgical pain. *Clin J Pain*. 2012;28(9):819-841.

How to cite this article: Hoofwijk DMN, van Reij RRI, Rutten BPF, et al. Genetic polymorphisms and prediction of chronic post-surgical pain after hysterectomy—a subgroup analysis of a multicenter cohort study. *Acta Anaesthesiol Scand*. 2019;00:1-11. <https://doi.org/10.1111/aas.13413>