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The Utility of Single Nucleotide DNA Variations as Predictors of Postoperative Pain

Gregory M. Jacobson^{1*}, Corinne J. Law², Harriet Johnston¹, Mark Chaddock³, Michal Kluger³, Raymond T.M. Cursons¹ and Jamie W. Sleight⁴¹Department of Biological Sciences, University of Waikato, Hamilton, New Zealand²Department of Anaesthesia, Anglesea Hospital, Hamilton, New Zealand³Waitemata DHB, Auckland, New Zealand⁴Waikato DHB, Hamilton, New Zealand**Abstract**

Objectives: Genetic variation is an important contributor to postsurgical pain and thereby analgesia requirements. A description of the potential predictive power of genetic variants in pain should instruct improvements in pain management postoperatively. We set out to examine whether a set of genetic variants in pain related genes would show any association with actual pain outcomes in a typical surgical population.

Methods: A candidate gene study was carried out in 135 surgical patients with 12 DNA variants (single nucleotide polymorphisms or 'SNPs') in known or putative pain pathway genes to detect associations with postoperative pain - measured by a verbal rating score (VRS) and patient-controlled analgesia (PCA) usage rate. Standard PCR based molecular biology approaches were used.

Results: At 20-24h after surgery, patients with the 1032G/1032G variant pair for the A1032G variant of the potassium channel *KCNJ6* gene had a slightly higher median VRS than those with 1032A/1032A or 1032A/1032G pairs ($p=0.04$; dominant genetic model). This small difference was most apparent in the orthopaedic surgery patients where the 1032G/1032G pair associated with VRS (median(interquartile range)) of 5(4-6) vs. 3(0.5-4) in 1032A/1032A or 1032A/1032G groups. For PCA, patients with 3435C/3435C or 3435C/3435T pairs for ATP-dependent efflux pump gene *ABCB1* variant C3435T used PCA at a considerably higher rate of 0.89(0.07-1.66) mg.h⁻¹ compared with just 0.11 (0-0.52) mg.h⁻¹ for the 3435T/3435T pair ($p=0.03$; dominant model). A significantly higher usage rate was also detected for opioid receptor *OPRM1* variant IVS2-691 with usage of 0.77(0.01-1.56) mg.h⁻¹ for the IVS2C/IVS2C or IVS2C/IVS2G group vs. 0.24(0-1.26) mg.h⁻¹ in the IVS2G/IVS2G group ($p=0.04$; recessive model).

Conclusion: While this study has identified some significant statistical associations the potential utility of the studied DNA variants in prediction of postoperative pain and patient-controlled opioid analgesia requirements appears to be quite limited at present.

Keywords: OPRM1; ABCB1; Postoperative; Pain; Morphine; Analgesia

Background

Pain is a complex trait influenced by psychological, social, physical and genetic factors (reviewed [1]). Of the various factors that influence postsurgical pain, genetic variation plays an important role, with many gene variants contributing to the pain phenotype. Their cumulative small effects occur through alteration of protein expression level or amino acid sequence and thereby protein functions. Accordingly, DNA variants, in particular single nucleotide polymorphisms (SNPs), may produce differences in perception of acute pain [2] or the efficacy of postoperative analgesia [3].

A number of studies have identified genetic associations with acute pain. A survey of the literature for these associations highlights: 1) the ATP-binding cassette (ABC) superfamily, subfamily B p-glycoprotein 170 (*ABCB1*) gene encoding an ATP-dependent drug efflux pump involved in multiple drug resistance. *ABCB1* is a major determinant of morphine bioavailability [4] and the 3435C allele of variant C3435T (rs1045642) in the gene associates with higher expression level and reduced morphine availability in the CNS. 2) The catechol-O-methyl transferase (*COMT*) gene encoding an enzyme that degrades several analgesic neurotransmitters, including noradrenaline and dopamine [7]. A well-studied variant in the gene, Val158Met (rs4680), encodes a valine-to-methionine variation that associates with reduced metabolic activity of the enzyme, as first reported by Scanlon and colleagues [8]. Reyes-Gibby [9] reports that cancer patients with the 158Val/158Val

genotype required 63% more morphine for efficient pain relief compared to patients with the 158Met/158Met genotype. Another group has found that the 158Met variant was actually associated higher pain scores during painful postsurgical procedures (with morphine analgesia) [10]. 5) The μ -opioid (*OPRM1*) gene encodes the μ -opioid receptor and is a very well-studied candidate gene for pain association [18,19]. Gene expression from the 118G allele of *OPRM1* variant A118G (rs1799971) - which codes for an asparagine to aspartic acid residue change- produces a receptor with reduced agonist-induced receptor signalling efficacy [20]. Various studies have shown association of this variant with increased pain scores and/or opioid analgesia requirements after surgery [21-24]. 6) The potassium inwardly-rectifying channel subfamily J, member 6 (*KCNJ6*) gene encoding an ATP-activated channel (GIRK2) that closes with increased intracellular ATP causing membrane depolarization and voltage-sensitive activation. GIRK2 is activated by several G-protein coupled receptors including opioid

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receptors [26]. Nishizawa and co-workers [27] report that two SNPs in the gene - A1032G (rs2070995) and G-1250A (rs6517442) associate with postoperative rescue analgesia requirements. 7) The sodium channel protein type 9 subunit alpha (*SCN9a*) gene encodes a voltage-gated channel involved in nociception [29]. For *SCN9a* variant Arg1150Trp (rs6746030), the channel protein expressed from the gene containing the 1150Trp variant shows enhanced levels of depolarizing activation [30].

Other studies have usually looked for associations of single DNA variants with chronic pain conditions, or for pain thresholds in laboratory tests of noxious stimuli on human volunteers. In this study we selected a panel of genetic variants, some with established and some with no previously suggested pain association, and measured whether this broader selection of DNA variants might contribute significantly to the variation in postoperative pain seen in a broad-based representative clinical sample of day-to-day adult postsurgical patients. We aimed to determine whether any of the DNA variants had real clinical usefulness for predicting high postoperative pain.

Methods

Clinical

We recruited participants who were undergoing elective non-emergent surgery (expected duration <180 min) and were aged 18-65 yr, were competent to consent, were able to comprehend the VRS and were able to activate the PCA device. Subjects with weight >120 kg and/or BMI >35, a preoperative diagnosis of malignancy, epilepsy, a neuromuscular disease, a previously documented psychiatric disease that required medication, pregnancy, a history of chronic opioid use or dependency or severe asthma (i.e. previously necessitating hospital admission), were excluded. Regional anaesthesia, neuraxial blockade and total intravenous anaesthesia was not permitted. Patients received standardised general anaesthesia consisting of an intravenous induction using propofol (1-3 mg.kg⁻¹), muscle relaxant, and maintenance with 0.7-1.3MAC desflurane. The intraoperative opioid dosing regimen was 2 mcg.kg⁻¹ fentanyl on induction, and a 2 mcg.kg⁻¹.h⁻¹ infusion from the time of surgical preparation to closure. All patients received 1g paracetamol, 40mg parecoxib, 4mg dexamethasone, 0.5mg droperidol intravenously intraoperatively. Local anaesthetic was infiltrated to the wound at skin closure. Intravenous morphine PCA was provided postoperatively to achieve satisfactory patient comfort (1.5 mg doses, 5 min lockout interval, and 8 mg.hr⁻¹ maximum dose). Protocols received approval of the New Zealand Northern X regional ethics committee (#NTX/09/06/047) with patients giving written informed consent.

Postoperative pain was quantified using a standard verbal rating scale (VRS) ranging from 0="no pain at all" to 10="the worst pain imaginable". The VRS was recorded upon awakening in the post anaesthetic care unit (post anaesthetic care unit - PACU; taken at rest) (VRS_{awake}) as well as for morning after surgery (at 20-24hrs) with activity (VRS_{diA}). For abdominal surgeries the pain score was for pain experienced during coughing and for orthopaedic surgeries the score was for pain during limb movement. Patient PCA usage rate over 20-24h post-surgery was also recorded (PCA_{mg,h}⁻¹).

Selection of DNA variants (SNPs)

In addition to the variants listed in the introduction (see points 1-7), and after examination of the literature, we chose to also examine: 1) an additional *ABCB1* gene variant, T1236C, which has been associated with differential transport of various drug substrates of the *ABCB1* efflux pump [6]; 2) an additional variant in *OPRM1*, IVS2-691,

which may encode an altered receptor protein [3,25]. 2) a variant in the potassium voltage-gated channel, delayed-rectifier, subfamily S, member 1 (*KCNS1*) gene, Ile40Val, associated with higher pain after noxious stimuli in healthy volunteers [28]); and finally: 3) variants of the cytochrome P450 oxidase *CYP3A4* or *CYP3A5* genes, namely C20230T in the *CYP3A4* gene, and A6968G in the *CYP3A5* gene.

DNA extraction

A 1 ml venous blood sample was collected at the time of surgical closure and stored in 4 ml of a DNA preservation solution (5M GITC, 20 mM sarkosyl, 30 mM trisodium citrate, 0.7% mercaptoethanol, pH 7). To extract the DNA, a 1 ml sample of this preparation was collected into a new tube and a 1/10th volume of 3 M sodium citrate and 0.5 ml of phenol-chloroform (pH 7) were added and the tubes centrifuged at 14K RCF for 15 min. The upper (aqueous) phase was collected and DNA precipitated by addition of an equal volume of isopropanol (AR grade), mixing and centrifugation at 14K RCF. The DNA pellet was washed in 70% ethanol and briefly air-dried before resuspension in between 50 and 200 µl of a storage buffer (TE buffer; 10 mM Tris, 1 mM EDTA, PH 8). DNA was quantified using a Nanodrop 2000 instrument (Thermo, NZ).

Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP)

The first enzyme reaction, to make copies of the DNA of interest (PCR), was performed i-Star Taq polymerase (Intron Ltd, USA) by initial strand separation at 95°C for 2 min followed by 35 repeats of temperature cycling between 95°C 30 s, 60°C 30 s, 72°C 30 s, and a final extension step for 5 min at 72°C. 100 ng of DNA was used in each reaction. Gene annotations, SNP reference numbers, SNP codes used in this report, DNA primer sequences and details of restriction enzyme test are shown in Table 1. Reagents (DNA primers) and specific enzyme-based tests for the variants (namely the restriction fragment length polymorphism method) were designed by us using SNP Cutter (http://bioapp.psych.uic.edu/SNP_cutter.htm) or WatCut (<http://watcut.uwaterloo.ca/watcut/watcut/template.php>) or taken from other published studies (as indicated in Table 1). The banding pattern from 10 µL of digested DNA products separated on a gel in an electrical field by electrophoresis at 100 V for 45 min was used to determine which DNA variants were present in each patient sample. Ten percent of the samples were analysed twice as a quality control.

Study design and SNP data quality controls

A minimum sample size of 128 individuals was required to achieve a statistical power of 0.9 for individual SNPs; assuming a medium effect size of 0.5, and a type I error rate of <0.05.

A test for suitability of the genetic tests (for Hardy Weinberg equilibrium) was carried out using an online calculator (<http://oege.org/software/hardy-weinberg.html>).

The measurement of pain intensity is complex. This is particularly evident in a plot of VRS against postsurgical PCA demands that shows many patients tolerating high VRS scores without increasing PCA usage (Figure 1). In this report we used statistical tests to measure potential influences of variants on VRS and PCA.

Statistical Methods

SNP association analysis

The VRS and PCA data were treated as ordinal. Briefly, for each SNP we analysed each genotype alone (i.e. the additive model, which

Gene	Symbol	SNP	Allele1/ position ^a / Allele2	Type/change	MAF (%) [†]	PCR primers	Size (bp)	Diagnostic fragments (bp)	Enzyme	Design
ATP-binding cassette, sub-family B, member 1	ABCB1	rs1045642	C3435T	Synon.	C 43	F AGCTGCTTGATGGCAAAGAAAT R TGGTCGAACACTTTCATCCCTT	433	T 387 C 236	Mbol	‡
		rs1128503	T1236C	Synon.	T 44	F TCTTTGTCACTTTATCCAGC R TCTCACCATCCCCTCTGT	502	C 361 T 416	HaeIII	§
Catechol-O- methyltransferase	COMT	rs4680	Val158Met	Non-synon.	A 39	F GTGGACGCGTGATTGAGGAG R CAGGCATGCACACCTTGTCGgTC	199	A 107 G 83	Tsp45I	‡
Cytochrome P450, family 3, subfamily A, polypeptide 4	CYP3A4	rs2242480	C20230T	Intronic	T 33	F ACCCTGATGTCAGCAGAAACT R ATAGAAAAGCAGATGAACCAGAGCC	284	C 216 T 284	Rsal	¶
Cytochrome P450, family 3, subfamily A, polypeptide 5	CYP3A5	rs776746	A6986G	Splice-3	A 3	F GCATAGGAGATACCCACGTATcT R TGGTCCAAACAGGGAAGAGgTA	118	G 97 A 118	Rsal	**
Neurobeachin1	NBEA	rs7990537	A11771G	Intronic	G 28	F CCCTTGGATGTTTGAACCTCTG R TTTCCTAGATGCCCTTCACTGG	433	A 433 G 332	Bts1	‡
Opioid Receptor, mu 1	OPRM1	rs1799971	A118G	Non-synon.	G 19	F TCAACTTGTCCCCTTAGATCGC R TGACCAGGAAGTTCCGAAGAG	180	A 180 G 158	BstUI	‡
		rs2075572	IVS2-691	Intronic	G 40	F TAGCTCTGGTCAAGGCTAAAgAT R CCCAGTACCAGGTTGGATGAGA	154	G 154 C 134	Mbol	‡
Potassium inwardly- rectifying channel, subfamily J, member 6	KCNJ6	rs2070995	A1032G	Synon.	A 20	F TAGAGGACCCCTCCTGGACT R CGGAACATCAGGCACAGTTT	298	A 197 G 175	MspI	‡
		rs6517442	G-1250A	Upstream	G 24	F TGGCTATTCTTGCTGCTTT R CAGTCATTTTAGAGGGCAGTCA	203	G 203 A 181	HinII	‡
Potassium voltage-gated channel, delayed-rectifier, subfamily S, member 1	KCNS1	rs734784	Ile489Val	Non-synon.	G 40	F AGTTTGAGGACTTGCTGAGCcGC R ATACATCTGAGGGTGTGGAGGC	137	A 137 G 115	BstUI	‡
Sodium channel, voltage- gated, type IX, alpha subunit	SCN9a	rs6746030	Arg1150Trp	Non-synon.	A 11	F GTTTTCCTGATGTTCCACCAGATT R TGAAGGAAGGCAGCATCCAATTA	244	A 244 G 172	Rsal	‡

Lower case in primer sequences indicates bases changed to alter restriction sites. Key: Type codes with protein level consequence: Synon.-synonymous, - no amino acid change; Non-synon.- non-synonymous, amino acid change as indicated; Intronic – variant occurs in a non-coding portion of the gene; upstream – in region upstream (5') of the gene; Slice-3 – causes transcript splice variation in 3' of the gene; IVS2 –within intron 2 of the gene (full SNP code:IVS2-691 G>C); UTR-3' - 3' untranslated region. † Minor allele frequencies (MAF) are from <http://www.ncbi.nlm.nih.gov/projects/SNP/>. ^aChromosome positions are from HumRef sequence and transcript positions are from Genbank NM reference sequences. Primers and test design ‡ (us); §[44], ¶[45], **[46].

Table 1: SNPs examined in this study: allele frequency, PCR primer sequences and RFLP test details.

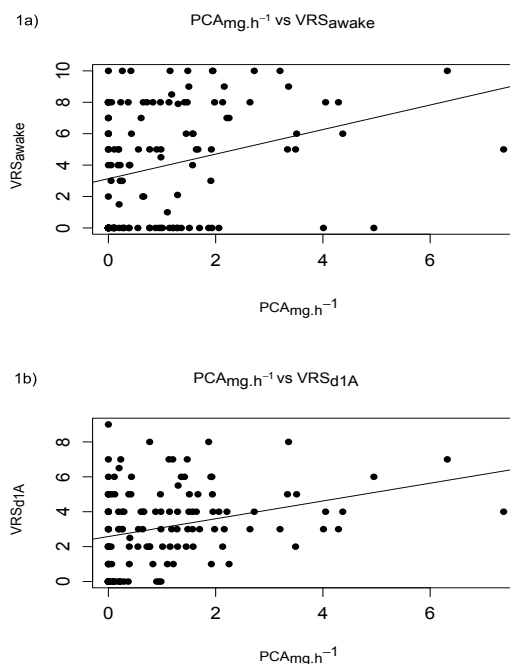


Figure 1: Scatterplots of VRS against PCA usage for all patients: A) VRS_{awake} vs. PCA_{mg.h⁻¹}; and B) VRS_{d1A} vs. PCA_{mg.h⁻¹}. Line of best fit is shown. Dots represent individual patients.

Basic information					
Age	43 yrs. (33-55)				
Gender	105♀/30♂				
Height	1.67m (1.6-1.73)				
BMI	27.5 (23.4-31.0)				
Caucasian	107 (79%)				
Polynesian	18 (13%)				
Asian	10 (8%)				
Surgery type	n	Duration (min)	VRS _{awake} median (IQR)	VRS _{d1A} median (IQR)	PCA _{mg,h-1} median (IQR)
All		86 (67-117)	4 (0-8)	3 (1-5)	0.49 (0-1.49)
General	54	86.5 (72-127)	0 (0-5)	2 (0-5)	0.39 (0-1.2)
Gynaecological	65	83 (54-113)	6 (3-5)	3 (2-4)	0.98 (0.12-1.9)
Orthopaedic	16	99.5 (73-152)	0 (0-8)	4 (1-6)	0.03 (0-0.6)

VRS_{awake} pain upon awakening; VRS_{d1A} VRS 20-24h postsurgery with light activity (e.g. coughing or movement); PCA_{mg,h-1} patient-controlled analgesia usage rate in mg.h⁻¹; BMI – body mass index. Where appropriate values are expressed as median(interquartile range).

Table 2: Demographics, surgery and pain data (n=135).

assumes that heterozygous individuals have a phenotype midway between the two homozygotes) and also grouped by the presence of either minor (less frequent) or major (more frequent) alleles (dominant or recessive models). For these models, where 'd' is the minor allele and 'D' is the major allele, the recessive model compared values from individuals with 'd/d' against 'D/D and D/d' genotypes and the dominant model compared values from individuals with 'D/D' against 'D/d and d/d' genotypes. This approach avoids assumptions regarding mode of inheritance and limits the chance of missing potentially interesting associations and was similar to a method described previously [32,33]. Nonparametric statistical approaches were used including: 1) the 1-way ANOVA (with Kruskal-Wallis Z test) to examine the independent effect of all non-SNP variables on VRS or PCA, and 2) the general linear model (NCSS software) to measure associations between SNPs and VRS or PCA. For all tests a p-value <0.05 was considered statistically significant.

Results

A total of 135 patients from two hospitals in the central North Island of New Zealand (Waikato DHB and Northshore DHB) were recruited into the study. Demographic data, surgery type and duration, and median VRS data are presented in Table 2. No statistically significant associations were found between any pain score or PCA usage and either age, ethnicity or gender. However there was a strong association between VRS_{awake} and surgery type (p < 0.001). To incorporate the influence of surgery type it was included in all SNP association tests for all outcomes using the general linear model (up to 2-way). Hence p values reported here are for the SNP-outcome with consideration of surgery type.

Some samples (<1%) failed to give a conclusive DNA test result and were excluded from further analysis. One *NBEA* variant (rs9565317) selected for inclusion in the study did not pass genetic testing (Hardy-Weinburg equilibrium) and was removed from the DNA variant panel. All results are expressed as median(interquartile range) unless otherwise stated.

Association Analysis

VRS_{awake}

No statistically significant SNP associations were found for VRS_{awake}.

VRS_{d1A}

An association between *KCNJ6* A1032G variant and VRS_{d1A} was

found (Table 3). Patients with the 1032G/1032G variant pair had higher median VRS scores when compared to patients with the 1032A/1032A or 1032A/1032G variant pairs (p=0.04; dominant model) (Table 3). The difference in VRS score was only apparent when the actual surgery type was examined (Table 5) and this weak association showed a VRS range of between 2 and 3 for 1032A/1032A or 1032A/1032G pairs and 2.3 to 5 for 1032G/1032G pairs (Table 4). No significant associations were found for any other DNA variants and VRS_{d1A} scores examined.

Patient-controlled analgesia usage rate (PCA_{mg,h-1})

A significant association between PCA usage rate and *ABCB1* gene variant C3435T was found (Table 3). For all surgery types individuals with 3435C/3435C or 3435C/3435T genotypes for C3435T consumed at a rate eight times faster in the same period - with median usage of 0.89 (0.07-1.66) compared to just 0.11 (0-0.52) mg.h⁻¹ in patients with the 3435T/3435T genotype (p<0.03; dominant model) (Table 5).

Also, for all surgery types combined, patients with the *IVS2C/IVS2C* or *IVS2C/IVS2G* genotypes for the *OPRM1* variant *IVS2-691* had markedly higher median PCA usage rates of 0.77(0.01-1.56) compared to 0.24(0-1.26) mg.h⁻¹ in patients with the *IVS2G/IVS2G* variant pair (Table 5). This association was weakest in the gynaecological patient group where patients with the *IVS2C/IVS2C* and *IVS2C/IVS2G* genotype had just a 10% higher PCA_{mg,h-1} than those with the *IVS2G/IVS2G* variant pair.

Discussion

The main findings of this study were that, in a broad-based sample of typical postoperative patients, a DNA variant (SNP A1032G in the *KCNJ6* gene) was associated with 20-24 h postoperative pain and two DNA variants (SNPs C3435T in *ABCB1* and *IVS2-691* in *OPRM1*) were associated with a higher PCA usage rate. As noted above, surgery type had a strong influence on VRS – this was statistically significant for VRS_{awake} – and surgery type was included in our statistical models.

We found that patients with two copies of the 1032G variant (i.e. 1032G/1032G) in *KCNJ6* had higher VRS_{d1A} when compared to individuals with the variant pairs 1032G/1032A or 1032A/1032A but this was only evident with consideration of the individual surgery types. Nishizawa [27] did not see any association between this variant and pain score but reported an association between the 1032A DNA variant and increased postoperative rescue analgesia requirements. They explain their result by stating that the alternative 1032G variant

Gene	SNP	Genotype	Count	%	VRS _{awake} (IQR)	p values			VRS _{d1A} (IQR)	p values			PCA _{mg,h} ⁻¹ (IQR)	p values		
						Addit.	Dom.	Rec.		Addit.	Dom.	Rec.		Addit.	Dom.	Rec.
ABCB1	C3435T	C/C	37	27.2	4 (0-8)	0.76	0.63	0.81	3 (1-5)	0.35	0.23	0.25	0.77 (0.2-1.5)	0.09	0.03	0.23
		C/T	63	46.3	5 (0-8)				3 (1-5)				0.9 (1-1.9)			
		T/T	36	26.5	4 (0-8)				3 (0.8-4)				0.11 (0-0.52)			
	T1236C	T/T	28	22.6	4 (0-8)	0.35	0.67	0.28	3 (5)	0.22	0.09	0.56	0.88 (0.11-2)	0.8	0.7	0.78
		C/T	62	50.0	5 (0-8)				3 (1-5)				0.69 (0-1.46)			
C/C		34	27.4	4 (0-8)	4 (2-6)				0.23 (0-1.23)							
COMT	Val158Met	G/G	34	25.4	5 (0-8)	0.35	0.51	0.42	3 (1-3.3)	0.97	0.89	0.92	0.59 (0.12-2.1)	0.15	0.72	0.05
		G/A	48	35.8	3 (0-6)				3 (2-5)				0.66 (0-1.3)			
		A/A	52	38.8	5 (0-8)				4 (1-4.5)				0.34 (0-1.66)			
CYP3A4	C20230T	A/A	9	6.6	5 (0-7)	0.17	0.88	0.26	2 (0-3.5)	0.78	0.91	0.61	0.26(0-1.5)	0.92	0.61	0.99
		A/G	38	27.7	3 (0-6)				4 (2-5)				0.715(0-1.5)			
		G/G	90	65.7	4 (0-8)				3 (1-5)				0.55 (0.06-1.54)			
CYP3A5	A6986G	A/A	5	3.7	0 (0-3)	0.57	0.18	0.32	0 (0-2)	0.25	0.23	0.06	0 (0-1.32)	0.33	0.24	0.38
		G/A	26	19.1	0 (0-6)				2 (1-4.5)				0.09 (0-1.32)			
		G/G	105	77.2	5 (0-8)				3 (2-5)				0.66 (0.1-1.57)			
NBEA	A11771G	G/G	1	0.9	5	0.22	0.72	0.43	1	0.93	0.88	0.46	0.39	0.73	0.46	0.75
		G/A	27	24.3	4 (0-8)				3 (1-5.5)				0.38 (0-1.36)			
		A/A	83	74.8	5 (0-8)				3(2-3)				0.55 (0.1-1.45)			
OPRM1	A118G	G/G	3	2.2	0 (0-8)	0.85	0.69	0.95	3 (2-4)	0.16	0.09	0.83	0.98 (0.37-1.2)	0.62	0.37	0.95
		A/G	31	22.6	5 (0-8)				3 (1-5)				0.77 (0-1.64)			
		A/A	103	75.2	4 (0-7)				3 (1-5)				0.49 (0-1.45)			
	IVS2-691	G/G	40	29.2	2 (0-5)	0.11	0.07	0.09	2 (0-4)	0.68	0.74	0.35	0.24 (0-1.26)	0.15	0.63	0.04
		G/C	59	43.1	5 (0-8)				4 (2-5)				0.72 (0-1.87)			
		C/C	38	27.7	5 (0-8)				3 (1-4)				0.77 (0.06-1.45)			
KCNJ6	A1032G	A/A	14	9.9	4 (0-6)	0.78	0.66	0.5	3 (1-5)	0.13	0.04	0.97	0.23 (0-1.59)	0.5	0.51	0.58
		A/G	58	41.1	4 (0-8)				3 (1-4)				0.6 (0-1.53)			
		G/G	69	48.9	5 (0-8)				3 (2-5)				0.52 (0.18-1.44)			
	G-1250A	G/G	20	14.6	5 (0-8)	0.2	0.07	0.55	1 (0-4)	0.1	0.27	0.2	0.1 (0-1)	0.49	0.59	0.27
		G/A	67	48.9	5 (0-8)				3 (2-5)				0.8 (0.12-1.57)			
		A/A	50	36.5	3 (0-7)				3 (1-5)				0.38 (0-1.5)			
KCNS1	Ile489Val	G/G	24	18.8	5 (0-8)	0.54	0.29	0.58	3 (1-4)	0.22	0.25	0.38	0.58 (0.12-1.53)	0.29	0.28	0.49
		A/G	58	45.3	5 (0-8)				3 (2-4)				0.69 (0.05-1.65)			
		A/A	46	35.9	3 (0-6)				3 (1-5)				0.22 (0-1.22)			
SCN9a	Arg1150Trp	A/A	0	0.0	n/a	0.37	0.37	n/a	n/a	0.28	0.28	n/a	n/a	0.06	0.06	n/a
		G/A	32	23.4	4 (0-7)				3 (1-5)				0.11 (0-0.9)			
		G/G	105	76.6	4 (0-8)				3 (1-4)				0.77 (0.1-1.56)			

Shaded cells with p values in italics, bolded and underlined indicate that a significant association (p<0.05) was detected. Addit. – additive model; Dom. – dominant model; Rec. – recessive model; IRQ – interquartile range.

Table 3: SNP frequencies, genotyping data and results of association analysis for all study subjects.

seems to confer an improved efficiency in mediating the transmission of opioid signaling compared with the 1032A variant. Another study found association between eight *KCNJ6* variants and postoperative oral analgesia usage but pain scores were not tested (nor were the A1032G or G-1250A variants) [34]. The exact function of these genetically synonymous *KCNJ6* variants is currently unknown but others suggest that the A1032G variant may regulate gene usage levels via mRNA secondary structure, with lower gene expression in individuals with the 1032A allele [27].

The association we describe between the *ABCB1* variant 3435T and lower PCA usage rate reflects the findings of another recent study that reports lower PCA requirements in individuals homozygous for the 3435T variant [5]. This effect is very likely due to the higher expression of the efflux pump that is driven from the 3435C variant [35,36]. Individuals carrying this allele therefore have a stronger barrier to opioids at the blood brain barrier so that less of the drug is available at receptors in the CNS [37] and a relatively higher PCA dose is required.

For the *OPRM1* variant IVS2-691, patients with IS2C/IS2C or IS2C/IS2G variant pairs had median PCA_{mg,h}⁻¹ pain levels more than two-fold higher than those with the IS2G/IS2G variant pairs across all surgery types. We could not find other studies linking the variant to variation in either postoperative pain or PCA requirements. However, the variant has been studied for effects on oral morphine efficacy in cancer patients although no association was found [38] and another study reported no association between the variant and morphine pharmacodynamics using plasma samples from healthy volunteers [39]. The IVS2-691 variant may exert the observed effects by causing variation in slicing patterns of mRNA transcripts or may be simply ‘tagging’ a nearby variant that actually confers some functional effect and is simply inherited during meiosis with the IVS2-691 variant due to proximity on the same chromosome.

Possible clinical applicability

In our patient cohort different DNA variants showed different associations for VRS (i.e. with *KCNJ6* gene variant) and PCA (i.e.

Gene	SNP	Model	Surgery	Groups	VRS (IRQ)	Count	p value
KCNJ6	A1032G	Dom.	All	1032A/A and 1032A/G	3(1-4)	64	0.18
				1032G/G	3(2-5)	67	
			General	1032A/A and 1032A/G	2 (0-5)	26	<u>0.04</u>
				1032G/G	2.3(1-4.3)	26	
			Gynaecological	1032A/A and 1032A/G	3(2-4)	29	
				1032G/G	4(2-5)	34	
			Orthopaedic	1032A/A and 1032A/G	3(0.5-4)	9	
				1032G/G	5(4-6)	7	

Dom. – dominant model; Rec. – recessive model. Underlined p value is for general linear model with 'surgery type' included.

Table 4: Details of significant association found between SNPs and VRS_{01A}.

Gene	SNP	Model	Surgery	Groups	VRS (IRQ)	Count	p value
ABCB1	C3435T	Dom.	All	3435C/C or 3435C/T	0.89(0.07-1.66)	100	0.02
				3435T/3435T	0.11(0-0.52)	36	
			General	3435C/C or 3435C/T	0.77 (0.12-1.29)	41	<u>0.03</u>
				3435T/3435T	0.09(0-0.39)	13	
			Gynae.	3435C/C or 3435C/T	1.22(0.19-1.94)	48	
				3435T/3435T	0.26(0-0.21)	17	
			Ortho	3435C/C or 3435C/T	0.06(0-1.42)	11	
				3435T/3435T	0(0-0.11)	5	
OPRM1	IVS2-691	Rec.	All	IVS2C/ IVS2G or IVS2C/ IVS2C	0.77(0.01-1.56)	96	0.03
				IVS2G/ IVS2G	0.24(0-1.26)	40	
			General	IVS2C/ IVS2C or IVS2C/ IVS2G	0.69(0.03-1.26)	36	<u>0.04</u>
				IVS2G/ IVS2G	0.24(0-1.05)	18	
			Gynae.	IVS2C/ IVS2C or IVS2C/ IVS2G	0.98(0.13-1.81)	49	
				IVS2G/ IVS2G	0.89(0.08-1.92)	16	
			Ortho.	IVS2C/ IVS2C or IVS2C/ IVS2G	0.06(0-1.42)	11	
				IVS2G/ IVS2G	0(0-105)	5	

Dom. – dominant model; Rec. – recessive model. IQR – interquartile range. Underlined p value is for general linear model with 'surgery type' included.

Table 5: Significant associations found between SNPs and PCAmg.h¹ with dominant or recessive models

with *ABCB1* and *OPRM1* variants). For VRS, the association with the *KCNJ6* variant was weak and is unlikely to have much clinical utility. PCA usage is a composite endpoint that involves a complex relationship including genetic effects acting on the intensity of the pain experienced by the patient and pharmacogenetic effects on the perceived potency of the morphine. Whether preoperative knowledge of genotypes that associated with PCA, for example the 3435T/3435T variant pair for the *ABCB1* gene (which had a >8-fold lower PCA usage rate and was found in around 26% of all study subjects), would allow the avoidance of postoperative PCA prescription is as yet untested. Such possible therapeutic interventions require formal testing in prospective studies. However, some groups have noted obstacles to adoption of this type of pharmacogenetic testing, including the unreliability of some of the evidence for associations due to weaknesses in the design of some candidate gene association studies and/or their irreproducibility [40]. Overlying these issues is the confounding factor of the subjective nature of pain perception [41].

Questions about the neuroscience of postoperative pain

While the *ABCB1* 3435C variant association with higher PCA usage rate for certain types of surgery agree with previous studies, the association for the *KCNJ6* A1032G variant with VRS was unexpected in light of evidence reported by Nishizawa [27]. Although it is possible that some associations we found evidence for may be statistical artefacts, we believe that our results call into question the simplistic pharmacokinetic and pharmacodynamic explanations of the genetic influences on pain and highlights the paucity of our knowledge of the interactions between nociceptive and pharmacological mechanisms.

One notable finding in our study was the lack of any association between the *OPRM1* A118G variant and any of the clinical outcomes. This may be due to a masking of the association due to the low percentage of Asian patients in the study (around 8%). Around 15% of the Asian population are minor allele homozygous for the variant (i.e. have the variant pair 118G/118G) compared to <2% in the Caucasian population. However, other studies have also failed to detect any association with the A118G variant and postoperative pain (VRS). Janicki [42] did not find any association for 118G with either pain score or morphine requirements following surgery and De Gregori [43] did not detect any association with PCA in a similar study (they did not examine pain score data).

Conclusion

We found evidence for various associations between DNA variants in *ABCB1*, *OPRM1*, and *KCNJ6* and postoperative pain and/or PCA usage. However, these results would appear to be more of scientific than clinical interest because the strength of the correlations do not appear to be strong enough to form the basis for useful clinical prediction.

Importantly, we found no pain association for examined variants in *COMT*, *CYP3A4*, *CYP3A5*, *KCNS1*, *NBEA* and *SCN9a* genes. This suggests that the particular variants studied in these genes are unlikely to have value in prediction of postoperative pain in a typical population of elective surgery patients. Future studies should focus on defining a more useful panel of DNA variants for reliable prediction of postoperative pain.

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Authors contributions

GJ selected relevant genes for the study, coordinated sample collection and processing, designed and carried out the RFLP tests and drafted the manuscript. CL assisted in study design, applied for ethics committee approval, recruited Waikato DHB patients, collated drug usage information, interviewed patients for relevant pain score information and helped to draft the manuscript. HJ helped design some RFLP tests, extracted DNA from patient samples and performed genotyping for some variants. MC and MK collected and collated Northshore DHB samples, assisted in study design and commented on the manuscript. RC participated in the design of the study and in data analysis. JS had the initial idea for the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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