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Nociceptive stimuli responses at different levels of general anaesthesia and genetic variability

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Background: Changes in skin conductance (SC), clinical stress score (CSS), the bispectral index spectroscopy (BIS) index and the variation in the BIS index may be used to monitor responses to nociceptive stimuli. We wanted to examine these methods during noxious stimulation during general anaesthesia and if the responses were associated with variability in genes related to pain.

Methods: Sixty patients, given propofol to a BIS level of 40–50, were stimulated with standardised tetanic electrical stimuli during propofol infusion, plasma level of 3 μg/ml alone, or together with remifentanil target plasma level of 3 ng/ml or 10 ng/ml. The CSS, SC, BIS index and the variability of the BIS index were registered. The inter-individual variation in nociceptive responses was analysed for co-variation with genotypes of 89 single nucleotide polymorphisms from 23 candidate genes.

Results: During tetanic stimuli, CSS and SC increased significantly and were attenuated with increasing level of remifentanil, different from the BIS index and the variation in the BIS index. Polymorphisms in the P-glycoprotein (ABCB1), tachykinin 1 receptor (TACR1), dopamine receptor D3 (DRD3) and beta arrestin 2 (ARRB2) genes were associated with the co-variation in SC variables or CSS response or both during standardised nociceptive stimuli ($P < 0.05$). Because of no corrections for multiple testing, the genetic analyses are explorative, and associations must be tested in further studies.

Conclusion: This exploratory study suggests genes that may be tested further with relation to nociceptive response during anaesthesia. SC and CSS may be useful tools for monitoring nociceptive response during general anaesthesia.

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General anaesthesia consists of hypnosis, analgesia and areflexia. The administration of hypnotic agents is used to prevent awareness, the analgesics to prevent autonomic and somatic responses, and muscle relaxants to prevent reflex movements. Different commercial devices have been developed to monitor depth of anaesthesia; like bispectral index spectroscopy (BIS), auditory evoked potential (AEP) and state entropy (SE). These are based on analyses of the Electroencephalography (EEG) signal and seem to be more related to the hypnotic state of the patient than to antinociception as perceived as pain or physiologic stress induced by noxious stimulation.[1]

Skin conductance (SC) mirrors the emotional part of the sympathetic nervous system, which is different from the part influencing the micro-circulation and the adjustment to temperature.[2] SC is correlated with changes in skin sympathetic nerve activity.[3,4] Skin sympathetic nerve activation results in filling of the palmar and plantar sweat glands, and then the SC increases transiently before the sweat is reabsorbed and the SC decreases. When a short-lasting outgoing sympathetic nervous burst occurs, a fluctuation of SC will follow.[3,4] An increase in the number of SC fluctuations (NSCF) can therefore be interpreted as increased skin sympathetic nerve activity.[3,4] Moreover, forceful bursts in the sympathetic nerves give rise to a huge area under the SC curve, area under the curve (AUC), and may be interpreted as increase in the arousal as seen, for example, during emergence from anaesthesia.[5] Functional magnetic resonance imaging of the brain and Visual Analogue Scale (VAS) during acute pain in awake volunteers demonstrates that the SC activity increases with pain-evoked brain

These results have been presented as abstracts at the International Society for Anaesthetic Pharmacology (ISAP) meeting in 2008 and the European Society of Anaesthesiology (ESA) meeting in 2009.

responses, consistent with a role of the SC activity to reflect pain-related autonomic processes.[6,7] Moreover, NSCF measures the response of noxious stimulation during tetanic stimulation, that is, strong electrical nerve stimulation of muscles, and the NSCF response is inversely related to the dose of opioids.[8] The NSCF is reflecting actions of acetylcholine as a neurotransmitter acting on muscarinic receptors[9,10] and is therefore not influenced by hypovolemia, adrenergic receptor active agents, room temperature changes or muscle-relaxing agents.[2,9–12] Moreover, NSCF reacts very fast, within 1–2 s, to nociceptive or painful stimuli.

It is well established through experimental studies in animal models and in human experimental studies that pain sensitivity is influenced by genetic disposition and that the proportional influence from genetic variability is related to the type of nociceptive stimuli.[13–15] Also, studies in patients suffering from chronic pain have suggested that variability within genes such as the GTP cyclohydrolase gene,[16] and beta-adrenergic receptor genes[17] may influence the nociceptive response. However, in the existing studies of awake humans, it is difficult to interpret whether the effect of genetic variability is associated with altered nociception or to changes in the emotional processing of pain or other parts of the pain experience.

The SC test represents an objective method to assess responses to standardised nociceptive stimulation in humans, both when awake and when unconscious.[8] During general anaesthesia, the responses will not be influenced by the patient's subjective experience. Therefore, in this preliminary study, we wanted first to test the clinical response like the clinical stress score (CSS),[5,8] the SC response, the BIS index and the variability of the BIS index for sensitivity to nociceptive stimulation. Second, we wanted to test if the measured inter-individual difference in response to standardised nociceptive stimulation was influenced by genetic variation in candidate genes known to be potentially related to pain sensitivity.

Method

Ethics

The study was performed at Oslo University Hospital, Ullevaal, in accordance with the principles of the Helsinki declaration and approved by the Regional Committee for Medical Research Ethics, Health Region I, Norway. The study was registered in Clin Gov Trials number NCT00327938.

All patients gave their oral and written informed consent before inclusion.

Subjects

Sixty women with American Society of Anesthesiologists physical status 1 or 2 with body mass index between 20 and 30 kg/sq.m were included. The women were scheduled for elective laparoscopic gynaecologic surgery. All patients had normal kidney function (serum creatinine concentration 60–105 micromole/l) and hepatic function (serum albumin concentration 36–45 g/l). Patients reporting either chronic pain during the last 6 months or abuse of analgesics were excluded. Only Caucasians[18] were studied to get a homogenous group of patients.

Experimental procedure

Premedication of 7.5 mg midazolam was given orally about 1 h before the surgery. After placement of an intravenous cannula, anaesthesia was induced with propofol with an effect-site target control infusion (TCI; Marsh model) at 3 μg/ml, and then adjusted until the bispectral index (BIS) (Aspect MS) was stable between 40 and 50. The smoothing of the BIS algorithm (BIS-Xp, Aspect MS, Boston, US, BIS A-2000, software version 3.12) was set to clinical standard of 30 s.

After induction of anaesthesia, but before intubation and start of the surgical procedure, the nociceptive stimulation tests were performed in three consecutive situations where the propofol was adjusted to keep BIS stable between 40 and 50 before each test:

1. No remifentanil,
2. Remifentanil effect-site concentration of 3 ng/ml, provided by an effect-site TCI, Minto model.[19,20]
3. Remifentanil effect-site concentration of 10 ng/ml.

Each target value of remifentanil was kept constant for at least 4 min before the tetanic stimulus was given (Fig. 1).

At each test experiment, the patients were stimulated with one tetanic stimulus using standard setup train-of-four (TOF) – guard stimulation of the median nerve with 50 mA for 30 s single impulse duration, square wave and frequency of 100 Hz at the TOF-guard (Life-tech, Stafford, TX USA, Model ES400). The patients response to this nociceptive stimulation was measured by the SC responses, changes in NSCF and the AUC, using the SC Algesimeter (Med-Storm Innovation, Oslo, Norway, version 060895), and the CSS (Table 1).[5,8] If the

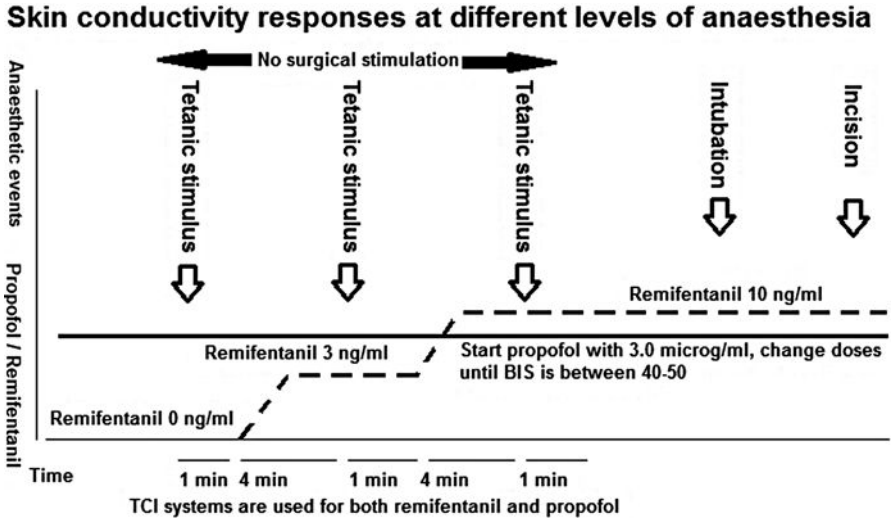

Fig. 1. Tetanic stimulation was performed three times with increasing doses of remifentanil. The patients were anesthetised with propofol to keep the BIS index between 40 and 50 before each tetanic stimulus.

Table 1

The clinical stress score (CSS).[5,8]

Criteria	
Large muscle movement	1
Coughing	1
Eye opening	1
Sweating in the forehead	1
Tears	1
Face muscle reaction	1
Systolic blood pressure > 130 mm Hg	1

For the CSS, 1 point of stress will be calculated for each of these reactions, adding all together to a total CSS. The CSS was estimated online by observing the patient.

patient started to move during the tetanic stimulation, the stimulation was stopped.

Blood samples for remifentanil analysis

To document the real plasma remifentanil concentration for anti-nociception, the patients had an arterial line established in the radial artery before the start of anaesthesia. Just before the start of the second and the third test of tetanic stimulation, arterial full blood samples were taken for analyses of remifentanil plasma concentration. Remifentanil in whole blood was analysed at Analytico Medinet, Breda, the Netherlands, using a liquid chromatography and mass spectrometry technique. The limit of quantification was 0.1 ng/ml ± 1, and the coefficient of variation was less than 15% in the range 0.1 ± 40 ng/ml.[21]

Blood samples and genotyping techniques

Arterial full blood samples for genotyping were obtained when sampling blood for remifentanil analyses before tetanic stimulus number 2. DNA was extracted from ethylene-diamine-tetra-acetic whole blood using the Gentra Puregene blood kit (QIAGEN Science, Germantown, MD, USA). Genotyping was performed using the SNPlex Genotyping System according to the supplier's dry DNA protocol (Applied Biosystems, Foster City, CA, USA). The capillary electrophoresis was carried out with an ABI 3730 48-capillary DNA analyser. SNPlex signals were analysed using the Gene Mapper v.4.0 software followed by manual reading. Samples giving low signals, which could not be discriminated from the negative controls, were removed prior to the analysis and treated as missing data. Two single-nucleotide polymorphisms (SNPs), rs4680 (*COMT*) and rs1045642 (*ABCB1*), which could not be analysed by the SNPlex system, were genotyped using TaqMan SNP allelic discrimination by means of an ABI 7900HT. The TaqMan SNP genotyping assays were quality-control tested and delivered in a convenient ready-to-run single-tube format. The choice of candidate genes was based on a clinical relevant frequency of the variant genes (allele frequency > 0.10) and a previously described association with or a putative functional effect to nociceptive responses. The genes and SNP markers assessed are listed in Table 2.

Statistics

In a previous study with similar clinical setup, 20 patients in each group provided significant differences in nociceptive responses.[8] In the present study with focus on unknown differences in genetic

Table 2

Genes and SNP markers tested for association with the nociceptive response.

Gene symbol	Gene product	SNP	Alleles
OPRD1	Opioid receptor, delta 1	rs533123	c/t
		rs678849	c/t
		rs2236857	a/g
TACR1	Tachykinin receptor 1	rs4439987	a/g
		rs6546952	c/t
		rs12475818	g/t
		rs3771836	g/t
		rs10191107	a/g
		rs12713837	c/g
		rs6725334	a/g
DRD3	Dopamine receptor D3	rs2399496	a/t
		rs9817063	c/t
		rs3732790	a/t
		rs3773679	a/g
		rs6280	c/t
		rs324026	c/t
ADRA2C	Adrenergic, alpha 2, receptor	rs1133450	g/t
		rs1133452	c/g
		rs1800037	c/g
UGT2B7	UDP glucuronosyltransferase 2 family, polypeptide B7	rs7668258	c/t
HINT1	Histidine triad nucleotide binding protein 1	rs4878	a/g
		rs11558046	g/t
		rs3852209	c/t
		rs2551038	c/g
		rs3864283	c/t
GABBR1	Gamma-aminobutyric acid (GABA) B receptor 1	rs10946999	g/t
		rs3025643	c/t
		rs29267	c/t
		rs740882	a/g
		rs29261	c/t
		rs29259	c/t
OPRM1	Opioid receptor, mu 1	rs1799971	a/g
		rs9479757	a/g
		rs540825	a/t
		rs562859	a/g
		rs548646	c/t
		rs1323042	a/c
		rs618207	c/t
		rs639855	g/t
		rs497976	a/c
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	rs1045642	a/g
		rs4437575	a/g
		rs2235013	a/g
		rs2235033	c/t
		rs1128503	c/t
		rs1202170	a/g
		rs7802773	a/g
		rs13229143	c/g
OPRK1	Opioid receptor, kappa 1	rs7815824	a/g
GABBR2	Gamma-aminobutyric acid (GABA) B receptor 2	rs2304389	a/g
		rs1435252	c/t
		rs2779562	c/t
		rs2808536	a/c
		rs3750344	a/g
ADRA2A	Adrenergic, alpha 2A, receptor	rs1800545	a/g
		rs13306143	a/g
		rs11195419	a/c
		rs3750625	a/c

Table 2

Continued

Gene symbol	Gene product	SNP	Alleles
TPH1	Tryptophan hydroxylase 1	rs1800532	a/c
DRD2		rs1554929	a/g
		rs6275	c/t
		rs7131440	c/t
		rs7122246	a/g
		rs17601612	c/g
		rs4274224	a/g
		rs7131056	a/c
HTR3B	5-hydroxytryptamine (serotonin) receptor 3B	rs11214763	a/g
		rs1176744	g/t
		rs1672717	c/t
		rs7943062	a/g
GNB3	Guanine nucleotide binding protein, beta polypeptide 3	rs5443	c/t
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	rs6312	a/g
		rs6311	c/t
GCH1	GTP cyclohydrolase	rs752688	c/t
		rs4411417	c/t
		rs3783641	a/t
MC1R	Melanocortin 1 receptor	rs1805009	c/g
ARRB2	Arrestin, beta 2	rs4790693	c/t
		rs16954146	a/g
		rs7208257	c/t
		rs1045280	c/t
OPRL1	Opiate receptor-like 1	rs6062627	g/t
COMT	Catechol-O-methyltransferase	rs2020917	c/t
		rs5993882	g/t
		rs4646312	c/t
		rs165722	c/t
		rs4633	c/t
		rs4680	a/g
GNAZ	Guanine nucleotide binding protein, alpha z polypeptide	rs3788339	a/g

variables, a patient population of 60 was chosen for the explanatory design. As potential trends and correlations for further more focused research was the scope of the present study, no primary endpoint was assigned, but a high number of candidate genes were tested.

The variables are given by mean and standard deviation (SD). To study how the different tetanic stimuli influenced the NSCF, AUC, CSS, BIS index, and variation in the BIS index, these variables were tested by Wilcoxon test between the 30 s prestimulus and the 30 s from the start of the tetanic stimulus. The SC values used were the NSCF and the AUC per 30 s analysing window. For the CSS and the BIS index value, the maximum values in each 30 s analysing window were used. The variation in the BIS index was measured as the variation in each 30 s analysing window by finding the minimum and maximum values. Then the differences of maximum and minimum value defined the range, and this difference was tested by Wilcoxon test. Moreover, when the responses during the tetanic stimuli were calculated, the values from before tetanic stimuli were deducted from the values after the tetanic stimuli for the different variables. The Wilcoxon test was used to test the differences of the responses between tetanic stimuli 1 and tetanic stimuli 2, and between tetanic stimuli 1 and tetanic stimuli 3. Furthermore, correlation tests between the CSS and the variables that proved sensitive to different analgesic levels were performed using the linear regression test. Moreover, the test variables that proved sensitive to different analgesic levels were tested against the polymorphisms for correlations with nociceptive responsiveness. For the exploratory analyses, Kruskal–Wallis test was used for polymorphisms in which three variants were observed, and Mann–Whitney *U*-test was used if only two genotypes were observed. The correlations are reported with *P*-values not corrected for multiple tests[22] because this is an exploratory study.

Table 3

Responses on tetanic stimulus for the skin conductance (SC) variables, number of skin conductance fluctuations (NSCF) and area under the curve (AUC), clinical stress score (CSS), the BIS index, and the variation of range of the BIS index, at different levels of analgesia. Tetanic stimulus 1 (Tet 1) no analgesia was given; tetanic stimulus 2 (Tet 2) remifentanil target = 3 ng/ml; and tetanic stimulus 3 (Tet 3) remifentanil target = 10 ng/ml.

Pre to post value during tetanic stimulus	Tet 1		Tet 2		Tet 3	
	Mean	*P*-value	Mean	*P*-value	Mean	*P*-value
NSCF/s	0.00 (0.00)–0.07 (0.07)	< 0.001	0.00 (0.00)–0.01 (0.03)	< 0.05	0.00 (0.00)–0.00 (0.00)	NS
AUC	0.00 (0.00)–40.00 (87.11)	< 0.001	0.00 (0.00)–0.11 (0.45)	< 0.05	0.00 (0.00)–0.00 (0.00)	NS
CSS	0.00 (0.00)–1.40 (0.92)	< 0.001	0.00 (0.00)–0.12 (0.37)	< 0.05	0.00 (0.00)–0.00 (0.00)	NS
BIS index	45 (8.6)–46 (11.4)	NS	44 (9.0)–48 (11.4)	< 0.001	44 (6.6)–45 (7.1)	NS
BIS variation	9.3 (6.5)–9.8 (6.3)	NS	7.0 (4.4)–9.4 (5.6)	< 0.001	7.0 (3.3)–7.3 (3.3)	NS

Table 4

Responses during tetanic stimulus 1 (Tet 1) was tested against the responses during tetanic stimulus 2 (Tet 2), and tetanic stimulus 3 (Tet 3) for number of skin conductance fluctuations (NSCF), skin conductance area under the curve (AUC), clinical stress score (CSS), the Bispaectral (BIS) index, and the variation of the range of the BIS index.

	Tet 1–Tet 2		Tet 1–Tet 3	
	Mean	*P*-value	Mean	*P*-value
NSCF	0.07 (0.7)–0.01 (0.03)	< 0.001	0.07 (0.07)–0.00 (0.00)	< 0.001
AUC	40.00 (87.10)–0.11 (0.45)	< 0.001	40.00 (87.10)–0.00 (0.00)	< 0.001
CSS	1.40 (0.92)–0.12 (0.37)	< 0.001	1.40 (0.92)–0.00 (0.00)	< 0.001
BIS index	1.2 (8.3)–3.8 (7.5)	NS	1.2 (8.3)–0.87 (4.3)	NS
BIS variation	0.5 (7.7)–2.4 (5.8)	NS	0.5 (7.7)–0.3 (3.1)	NS

The statistical software SPSS for Windows XP version 16.0 (IBM, Oslo, Norway) was used for all statistical analyses.

Significant *P*-value was defined to be $P < 0.05$ for the variables of clinical and physiological monitoring.

Results

Patients and anaesthetic procedure

We included 60 females scheduled for gynaecologic laparoscopic surgery, ASA group 1–2. Mean body mass index was 24.6 (± 4.3) kg/m^2, [mean (± SD)], and the mean age was 42 (± 19.9) years. Eight of the 60 women smoked cigarettes daily. In four patients, an intra-arterial line was not possible to establish, and for these patients, no blood sample for remifentanil analysis was taken. The measured remifentanil plasma concentrations [mean (± SD)] during tetanic stimulation number 2 (TCI remifentanil of target = 3 ng/ml) and tetanic stimulation number 3 (TCI remifentanil of target = 10 ng/ml) were 2.10 (± 0.54) ng/ml and 8.18 (± 2.42) ng/ml, respectively.

SC, BIS index and clinical stress responses

In two patients, the SC monitor registrations were discarded due to technical problems; the other 58 registrations were successful. The NSCF and AUC during tetanic stimulation 1 (i.e. no remifentanil) showed a significant more forceful reaction in 67% of the patients, compared with during tetanic stimulation number 2 (remifentanil TCI target = 3 ng/ml), whereas a reduced SC reaction occurred in only 8% of the patients. During tetanic stimulus 3 (remifentanil TCI target = 10 ng/ml), no reaction in NSCF and AUC occurred in any of the patients (Tables 3 and 4). Registration from one representative patient is showed in Fig. 2.

During tetanic stimuli 1 and 2, statistically significant increases in the NSCF and AUC were seen when compared with the pre stimuli levels (Table 3).

CSS increased extensively in 87% of the patients during tetanic stimulus 1 compared with during tetanic stimulation 2 with significant less increase in the clinical stress response, occurring in only 12% of the patients. No change was seen in CSS during tetanic stimulus 3 (Tables 3 and 4). During tetanic stimuli 1 and 2, a statistically significant increase in the CSS was seen when compared with the baseline without stimuli (Table 3).

There was a positive correlation between the SC responses, peaks per sec (NSCF) and the AUC, and the CSS, $r = 0.53$ and $r = 0.44$ ($P < 0.001$), respectively.

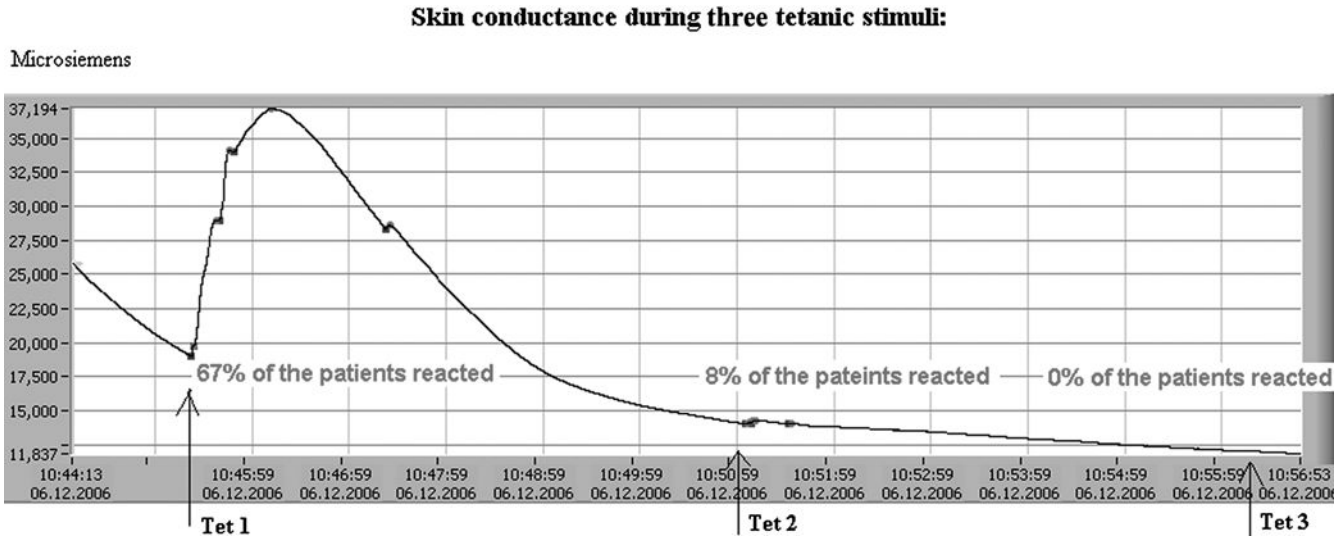

Fig. 2. Skin conductance (SC) response from one typical patient. During tetanic stimulus 1, that is, no remifentanil, forceful SC reaction is seen. During tetanic stimulus 2, target of 3 ng/ml remifentanil, less SC reaction was observed. During tetanic stimulus 3, target of 10 ng/ml remifentanil, no SC reaction was observed. Still, a huge variation between individuals was seen, 67% of the patients reacted according to the SC without remifentanil, but only 8% of the patients reacted with 3 ng/ml remifentanil, and none of the patients reacted when 10 ng/ml remifentanil was given.

The maximum BIS index and the variation in the BIS index increased significantly only during tetanic stimulation 2 when compared with the baseline without stimuli (Table 3). No statistical significant differences were seen between the maximum BIS index and the variation in the BIS index when the reaction during tetanic stimulation 1 was compared with tetanic stimulation 2 and 3 (Table 4).

Genotypes

The candidate genes and SNP markers assessed are listed in Table 2. For all SNPs, the distribution of genotypes was in Hardy–Weinberg equilibrium. The SNPs rs7815824 (*OPRK1*), rs16954146, rs7208257 (*ARRB2*), rs6312 (*HTR2A*), rs1805009 (*MC1R*), rs13306143 and rs3750625 (*ADRA2A*) were observed to have low frequencies of variant alleles (allele frequency < 0.10) and were not analysed further. The remaining genotypes were analysed with respect to their ability to predict the SC variables and CSS after tetanic stimulation number one, which was performed without analgesics. SNPs in the *ARRB2* gene (rs1045280), *ABCB1* gene (rs1128503), *TACR1* gene (rs6546952) and *DRD3* gene (rs3773679) were associated with the SC NSCF and/or the SC AUC (Table 5) at a $P < 0.05$ level, not corrected for multiple tests. SNPs in the *ARRB2* gene (rs1045280) and *TACR1* gene (rs6546952 and rs10191107) were also similarly associated with the CSS.

Discussion

This study showed that, in contrast to BIS and the variation of the BIS index, the SC variables (NSCF and AUC) and CSS are sensitive to tetanic noxious stimulation during anaesthesia, and that these measured responses are attenuated and negatively correlated to the measured plasma level of an opioid analgesic. In addition, the study suggests that SC response and the CSS to nociceptive stimuli are associated with the genetic variability in genes believed to influence pain sensitivity.

The model applied in this study is special compared with most other experimental and clinical pain models as it applies standardised nociceptive stimuli delivered to anaesthetised patients. Therefore, this clinical experimental model may study human nociceptive processing isolated from the pain experience, which by definition is the individuals' subjective experience of pain following nociceptive stimuli. Pain is a complex experience resulting from the physiologic response of the nociceptive stimuli but also influenced by other factors, such as the individual previous personal experiences and the context in which pain is experienced. Therefore, a model in which nociceptive processing could be isolated from the total pain experience may be of value.

The explorative analysis of genes that may be associated to nociceptive sensitivity revealed that

Table 5

Relation between genotypes and responses to tetanic stimulus for number of skin conductance fluctuations (NSCF), skin conductance area under the curve (AUC), and clinical stress score (CSS). All values are given as mean (standard deviation). *P*-values are for comparisons across genotypes. Only the genotypes that obtained statistical significance are presented.

	Genotype	Skin conductance NSCF		Skin conductance AUC		CSS	
ARRB2	TT ($n = 30$)	0.09 (0.06)	$P = 0.026$	52.0 (94)	$P = 0.048$	1.8 (0.9)	$P = 0.006$
rs1045280	TC ($n = 20$)	0.04 (0.05)		36.0 (91)		1.0 (0.8)	
($n = 56$) beta arrestin 2	CC ($n = 6$)	0.07 (0.13)		3.5 (8)		1.0 (0.8)	
ABCB1	CC ($n = 15$)	0.09 (0.06)	$P = 0.045$	46 (93)	$P = 0.441$	1.6 (0.9)	$P = 0.554$
rs1128503	CT ($n = 21$)	0.06 (0.08)		53 (105)		1.3 (1.0)	
($n = 43$), P-glycoprotein	TT ($n = 7$)	0.08 (0.06)		43 (104)		1.7 (0.9)	
TACR1	TT ($n = 19$)	0.10 (0.04)	$P = 0.061$	74 (109)	$P = 0.032$	2.0 (0.60)	$P = 0.033$
rs6546952	TC ($n = 24$)	0.06 (0.06)		44 (102)		1.3 (0.94)	
($n = 55$) tachykinin 1 receptor	CC ($n = 12$)	0.07 (0.08)		12 (30)		1.2 (0.93)	
TACR1	GG ($n = 4$)	0.08 (0.05)	$P = 0.13$	107 (158)	$P = 0.11$	2.0 (0.81)	$P = 0.024$
rs10191107	AG ($n = 30$)	0.09 (0.07)		31 (74)		1.7 (0.86)	
($n = 57$) tachykinin 1 receptor	AA ($n = 23$)	0.05 (0.06)		41 (88)		1.0 (0.89)	
DRD3	GG ($n = 23$)	0.05 (0.05)	$P = 0.046$	99 (140)	$P = 0.048$	1.4 (0.8)	$P = 0.55$
rs3773679	AG ($n = 26$)	0.10 (0.08)		28 (65)		1.6 (0.9)	
($n = 57$) dopamine receptor D3	AA ($n = 8$)	0.05 (0.05)		33 (83)		1.3 (0.9)	

SNPs in the *ARRB2* gene (rs1045280), *ABCB1* gene (rs1128503), *TACR1* gene (rs6546952) and *DRD3* gene (rs3773679) were associated with responses to tetanic nociceptive stimuli as measured by changes in SC and/or CSS. The preliminary nature of our findings must be underlined. The analyses did not correct for multiple analyses[22] and did not replicate the findings in other patient cohorts.[23] As we did not know which specific genes were of highest interest, we chose to include a large number of candidate genes. This will not only reduce the chance of missing an important gene but, by pure chance, create some *P*-values below 0.05 without necessarily reflecting a true correlation in the population. Furthermore, the absence of a gene-dose effect in some of the SNPs further stresses the need to interpret the findings carefully. Thus, our results should be interpreted with great caution, and no final conclusions should be made. However, the SNP markers, observed to be associated with responses to nociceptive stimuli in this study, may identify candidate genes that may be of interest to include in the future more specific and better powered studies.

Remifentanil was not given during the nociceptive stimuli used for the genetic analyses. Thus, genetic variability related to differences in opioid efficacy does not influence these results.

The genetic polymorphisms may be linked with an extensive cortical network associated with pain processing. This network includes, at least, the anterior cingulate cortex, the agranular insular cortex, the primary and secondary somatosensory cortices, the ventrolateral orbital cortex, and the motor cortex.[24] These cortical structures constitute the medial and lateral pain systems, the nucleus submedius-ventrolateral orbital cortex periaqueductal grey system, and motor cortex system, respectively. Multiple neurotransmitters, including opioids, glutamate, GABA and dopamine, are involved in the modulation of pain by these cortical structures.[24] Interestingly, injection of dopamine 2 like (D2/D3) agonists into ventrolateral orbital cortex produced dose dependent antinoception that was antagonised by dopamine D2/D3 receptor antagonists.[25] Dopamine D2/D3 activation may inhibit the inhibitory action of GABAergic inter neurons on the output neurons projecting to periaqueductal grey leading to activation of the brainstem descending inhibitory system and depression of nociceptive inputs at the spinal dorsal horn.[25] Moreover, the genetic variability in the dopamine beta-hydroxylase, the enzyme responsible for synthesis of noradrenaline from dopamine may induce chronic pain and hyperalgesia.[26] Polymorphism in the *ABCB1* gene, encoding the multidrug efflux transporter P-glycoprotein, is shown to be associated with pain relief by morphine.[27] Moreover, P-glycoprotein has been found to influence neurotoxicity and therefore be of importance in the treatment of neuropathic pain.[28] The neurotransmitter substance P modulates sensitivity to pain by activating the tachykinin 1 receptor, which is expressed by discrete populations through the central nervous system. Peripheral nerve injury increases the expression of tachykinin 1 receptor in the spinal cord, dorsal horn; this is correlated with heat hyper-

sensitivity.[29] Beta-arrestin 2 plays important physiological roles in regulating the function of the mu opioid receptors.[30]

It is known that the genetic variation in the serotonin transporter gene (*SLC6A4*, rs25531) influences the analgesic response to the short acting opioid remifentanil in humans.[31] However, propofol was used during the nociceptive stimuli for genetic analyses. Propofol is metabolised mainly by glucuronidation by uridine diphosphate-glucuronosyltransferases and by hydroxylation by CYP2B6 and CYP2C enzymes,[32] but these genes are not known to influence pain perception.

Genes controlling human sweat production are known to cause variation during some specific diseases. Cystic fibrosis has a mutation of the gene encoding for cystic fibrosis transmembrane conductance regulator,[33] ectodermal dysplasia have a mutation of *x*-linked hypohidrotic ectodermal dysplasia,[34] trancient aquagenic palmar hyper wrinkling (TAPH),[35] and the recessive phenotype of deafness mutant Connexin26M34T.[36] However, such variations are very rare and unlikely to influence the results in an unselected, healthy population. The SC changes are unique because, differently from responses measured by EEG monitors, they are immediate and show the individual response objectively in real time. Our method is validated previously using tetanic stimulus together with SC, SE and response entropy. Only the SC response decreased when the level of analgesia increased, differently from the EEG responses,[8] which is in accordance with the observations in this study. Moreover, the change of NSCF followed changes in norepinephrine blood levels during tracheal intubation, differently from the BIS index, arterial blood pressure and heart rate.[37] The sensitivity and specificity of the SC score to discover nociceptive responses pre-operatively were about 90% when a CSS was used as a reference.[5] Moreover, the NSCF have been used to evaluate pain responses in preterm infants,[38] artificially ventilated children,[39] post-operatively[40–42] and during perioperative stress.[5,37]

In this study, intra-arterial line was used to obtain blood pressure measure in real time, and no neuromuscular blocker was used. The CSS was therefore sensitive in detecting response to tetanic stimulus. Further studies are needed to see if the CSS is more sensitive than the SC device if the blood pressure is measured non-continuously or neuromuscular blockers are used.

Furthermore, the BIS index and the variation in the BIS index obtained statistical increase only during tetanic stimulus 2, not during tetanic stimulus 1 or 3. During tetanic stimulus 1, the patients often started to move and then the stimulation was stopped before 30 s and registrations not fulfilled. According to Rantanen et al., the EEG monitors need stimulus period of 30 s to react fully,[43] which may explain the lack of an increase in BIS score from baseline during tetanic stimulus number 1 in this study. Moreover, it seems like the increase in BIS index or the variation in the BIS index have low sensitivity when monitoring changes in nociception,[44] as the response during remifentanil infusion (tetanic stimulus 2 and 3) was not different from the response without opioids (tetanic stimulus 1) (Table 4). Further, the range of the BIS index in different patients before tetanic stimulus 1 was as high as 30. The index is quite variable even when a range of 40–50 is aimed for without any stimuli.

In this study, we focused on nociceptive sensitivity in anaesthetised patients, which is correlated with pain tolerance in the awake patients. The principal ascending pathways for nociception originate in the dorsal horn of the spinal cord. Control and awake sensitivity to pain may also reside in additional neurological loci, especially in the mesolimbic system of the brain (i.e. a reward centre), and a number of genes and associated polymorphisms may indeed impact pain tolerance and or sensitivity.[45] These polymorphisms may be associated with a predisposition to intolerance or tolerance to pain.[46] Traditional twin studies using various models of human experimental pain confirm that genetic factors contribute to some of the variability in nociception and perceived pain sensitivity.[47] The importance of genetics in pain sensitivity is also supported by ethnic differences in humans.[48] The genetically mediated variability varies between pain modalities both in terms of the extent of genetically explained influence and in the genetic factors involved for each pain modality.

The balance of pain or nociceptive stimulation versus analgesic dose effect may be a major focus for further research. Pre-operative pain response to noxious stimulation has previously shown to be correlated with the degree of early post-operative pain.[49] With tetanic stimulation in anaesthetised patients, the increase in the SC variables was significantly associated with the genetically modulated pain sensitivity. Changes in the SC variables may therefore be a sensitive and specific tool for predicting the need for analgesia during surgery as well as after surgery, and thus the risk of developing post-operative pain.[50] The SC variables can be useful in

tailoring the need for analgesics and may allow taking greater advantage of short-lasting analgesics by assisting in adequate titration of drug doses.

To conclude, we present a human experimental model where nociception can be studied in the non-awake patients. We also report, in this preliminary explorative analysis, that polymorphisms within the *ARRB2* (beta arrestin), *TACR1* (tachykinin 1 receptor), *ABCB1* (P-glycoprotein) and *DRD3* (dopamine receptor D3) genes may be associated with nociception as measured by changes in SC and/or CSS. These genes can be regarded as candidate genes for nociceptive response and should be further investigated in future studies.

Conflict of interest: Hanne Storm is also the chief executive officer and co-owner of Med-Storm Innovation that has developed the SC equipment used in this study. Med-Storm Innovation sponsored Oslo University Hospital-Ullevaal, with the SC equipments from Med-Storm Innovation when this study was performed at the hospital. Part of Hanne Storm's University work is expected to be research. Her research field has the last years been to monitor SC activity during pain and nociceptive stimuli.

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