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Pain

Chronic postsurgical pain in the Evaluation of Nitrous Oxide in the Gas Mixture for Anaesthesia (ENIGMA)-II trial

M. T. V. Chan[1]*, P. J. Peyton[2,3], P. S. Myles[4,5,6], K. Leslie[6,7,8,9], N. Buckley[10], J. Kasza[6], M. J. Paech[11,12], W. S. Beattie[13,14], D. I. Sessler[15], A. Forbes[6], S. Wallace[4,5], Y. Chen[1], Y. Tian[1], W. K. K. Wu[1,16,17] and the Australian and New Zealand College of Anaesthetists Clinical Trials Network for the ENIGMA-II investigators†

[1]Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China, [2]Department of Anaesthesia, Austin Hospital, Melbourne, Victoria, Australia, [3]Department of Surgery, University of Melbourne, Melbourne, Victoria, Australia, [4]Department of Anaesthesia and Perioperative Medicine, The Alfred Hospital, Melbourne, Victoria, Australia, [5]Department of Anaesthesia and Perioperative Medicine, Monash University, Melbourne, Victoria, Australia, [6]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia, [7]Department of Anaesthesia and Pain Management, Royal Melbourne Hospital, Melbourne, Victoria, Australia, [8]Anaesthesia, Perioperative and Pain Medicine Unit, and, [9]Department of Pharmacology and Therapeutics, University of Melbourne, Melbourne, Victoria, Australia, [10]Department of Anesthesia, Michael G. DeGroote School of Medicine, McMaster University, Hamilton, Ontario, Canada, [11]Department of Anaesthesia and Pain Medicine, Royal Perth Hospital, Perth, Western Australia, Australia, [12]School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, Australia, [13]Department of Anesthesia, University of Toronto, Toronto, Ontario, Canada, [14]Department of Anaesthesia, Toronto General Hospital, Toronto, Ontario, Canada, [15]Department of Outcomes Research, Anesthesiology Institute, Cleveland Clinic, Cleveland, OH, USA, [16]Li Ka Shing Institute of Health Sciences, Institute of Digestive Disease, Chinese University of Hong Kong, Hong Kong Special Administrative Region, China and [17]State Key Laboratory of Digestive Disease, Chinese University of Hong Kong, Hong Kong Special Administrative Region, China

*Corresponding author. Email: mtvchan@cuhk.edu.hk

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Abstract

Background. Previous animal and clinical studies showed that nitrous oxide may produce long-term analgesia. The aim of this study was to evaluate the effect of nitrous oxide in preventing chronic postsurgical pain. We also explored whether methylenetetrahydrofolate reductase gene polymorphisms (1298A>C, 667C>T) would enhance nitrous oxide analgesia.
Methods. We conducted a telephone interview at 12 months after surgery on 2924 (41.1%) patients enrolled in the Evaluation of Nitrous Oxide in the Gas Mixture for Anaesthesia-II trial. Pain at the wound site was recorded using the modified brief pain inventory and the neuropathic pain questionnaire. General health status was measured using the EQ-5D questionnaire. Genotyping was performed in a subset of 674 Asian patients in Hong Kong.
Results. At 12 months after surgery, 356 (12.2%) patients reported chronic postsurgical pain at the wound site and 112 (3.8%) patients had severe pain and required regular analgesic interventions. Nitrous oxide did not affect the rate of chronic postsurgical pain (11.8% nitrous oxide group; 12.5% no nitrous oxide group), relative risk (95% confidence intervals): 0.94 (0.75–1.17), P=0.57. However, in a planned subgroup analysis, nitrous oxide reduced the risk of chronic postsurgical pain in Asian patients, relative risk (95% confidence intervals): 0.70 (0.50–0.98), P=0.031. Patients who were homozygous for either gene polymorphism and who received nitrous oxide during surgery were less likely to report chronic postsurgical pain.
Conclusions. Nitrous oxide administration had no impact on chronic postsurgical pain, but benefits may still be possible in Asian patients and patients with variants in methylenetetrahydrofolate reductase gene.
Clinical trial registration. NCT00430989.

Key words: chronic pain; ; nitrous oxide; ; polymorphism; , single nucleotide; ; surgery

Editor's key points

- Perioperative nitrous oxide may have a role in preventing chronic pain after surgery.
- This large multicentre trial evaluated patients 1 yr after surgery using structured telephone interviews.
- Overall, ~12% of patients experienced chronic pain, with or without nitrous oxide treatment.
- In a subgroup of Asian patients, nitrous oxide reduced chronic pain risk, dependent on genotype.

Chronic postsurgical pain is defined as persistent pain over the surgical site for at least 2 months after surgery.[1] Depending on the type of procedure, the reported incidence of chronic postsurgical pain ranges from 10% after Caesarean section to 30–50% after limb amputation.[2–6] Given that quality of life and physical activities are adversely affected,[4 7 8] a strategy that prevents chronic postsurgical pain is therefore highly desirable.

Emerging data suggest that nitrous oxide might reduce the risk of chronic postsurgical pain. In rat models of inflammatory pain, 50% nitrous oxide inhibited the *N*-methyl-D-aspartate receptor, reduced pain sensitization, and prevented delayed hyperalgesia.[9 10] In addition, by an irreversible inhibition of methionine synthase, nitrous oxide blocks both the folate and methylation cycles, resulting in misincorporation of nucleotides, DNA damage, and demethylation.[11 12] Each of these processes may influence pain gene transcription. Interestingly, two functional polymorphisms in the methylenetetrahydrofolate reductase gene (1298A>C and 667C>T) block the folate cycle[13 14] and have been shown to exaggerate the biochemical effects of nitrous oxide (Supplementary Fig. S1).[15] Taken together, genetic predisposition may further enhance long-term analgesia with nitrous oxide administration.

Despite the encouraging animal data, no study has confirmed long-term analgesia from nitrous oxide in humans. In a single-centre, long-term follow-up study of 423 patients recruited to the Evaluation of Nitrous Oxide in the Gas Mixture for Anaesthesia (ENIGMA) trial from Hong Kong,[16] we showed that administration of nitrous oxide during anaesthesia reduced the risk of chronic postsurgical pain [7.0 *vs* 14.8%, adjusted odds ratio (95% confidence interval, CI): 0.48 (0.33–0.93), P=0.04].[17] However, the study was limited by the small sample size, and further investigation is required to confirm this finding. We have therefore prospectively designed and conducted a long-term follow-up study, among patients who were enrolled to the ENIGMA-II trial,[18] to evaluate the impact of nitrous oxide administration on persistent wound pain at 12 months after surgery. As a secondary aim, we tested the interaction between variants in the methylenetetrahydrofolate reductase gene and nitrous oxide administration on the development of chronic postsurgical pain.

Methods

The ENIGMA-II trial was a randomized controlled trial that evaluated the effect of nitrous oxide on perioperative cardiovascular complications in 7112 at-risk patients having major non-cardiac surgery. The trial objectives and study protocol are reported in a methods paper[19] and are summarized in a trial registry (ClinicalTrials.gov identifier: NCT00430989). Results on the risks of death and cardiovascular complications or surgical site infection within 30 days after surgery were similar among patients receiving nitrous oxide or not and have been reported previously.[18]

Patients

The ENIGMA-II patients were 45 yr or older, with risk factors for perioperative cardiovascular complications. All patients had general anaesthesia for non-cardiac surgery that was expected to last for 2 h or longer. We excluded patients who were to undergo thoracic surgery that required one-lung ventilation. Patients were also excluded if they had significant impairment in lung gas exchange and were planned to receive high concentrations of oxygen (>50%) during anaesthesia or if they reported past experience of severe postoperative nausea and vomiting.

Procedures

Patients were randomly assigned to receive 70% nitrous oxide in 30% oxygen or 70% nitrogen in 30% oxygen during anaesthesia. Randomization was stratified by site with permuted blocks. All other aspects of perioperative care, including the use of regional block and perioperative analgesia, were left to the discretion of the attending anaesthetists and surgeons. Death and major cardiovascular complications, including non-fatal myocardial infarction, cardiac arrest, pulmonary embolism, and stroke within 30 days of surgery, were recorded. Patients, surgeons, and the assessors for all outcome measures were blinded to treatment allocation.

Long-term follow-up

Before the start of the trial, the steering committee planned a 12 month follow-up study to evaluate the long-term effect of nitrous oxide on disability, stroke, myocardial infarction, and death. A protocol amendment was submitted in February 2010 and was subsequently approved by the ethics committees of all participating centres to undergo additional evaluation of chronic postsurgical pain. Follow-up was conducted using medical record review and structured telephone interview. The present report is restricted to the chronic pain outcomes; other long-term outcomes have been reported previously.[20]

During the telephone interview, patients were asked if, in the last 3 months, they had experienced pain in the surgical wound from the index surgery. When patients answered yes, they were further questioned specifically about whether the pain had been present before their surgery.[21] When they reported that the pain had developed since the operation, the presence of chronic postsurgical pain was recorded, and the severity, its impact on daily function, and characteristic of pain were rated using the modified brief pain inventory and the neuropathic pain questionnaire, respectively.[22–24] General health status was recorded in all patients using the EQ-5D questionnaire.[25]

The primary outcome was the presence of chronic postsurgical pain as defined by the International Association for the Study of Pain.[1] The secondary outcome was severe pain defined as average pain throughout the previous 24 h rated by the patient at $\geq$50 of 100 points.[7 17]

Genotyping

In a subgroup of Asian patients recruited from Hong Kong, 5 ml blood samples were collected before surgery. DNA was extracted using a standard phenol–chloroform protocol[26] and genotyped for methylenetetrahydrofolate reductase gene variants 1298A>C and 667C>T using the Taqman genotyping assays (Life Technologies, Carlsbad, CA, USA). This was performed using 384-well plates together with Taqman genotyping master mix (Life Technologies) on a 7900HT fast real-time polymerase chain reaction system (Life Technologies).

Statistical analysis

Assuming 12% of patients reported chronic postsurgical pain, an enrolment of 2924 patients would provide 83% power to detect a relative risk reduction of 25% with a type I error of 0.05. Data were analysed on an intention-to-treat basis. We reported differences in outcomes between groups as risk ratios with 95% CIs using binary regression with a logarithmic link. Patients not assigned to nitrous oxide were used as the reference group. We assessed the differences in outcomes in a number of prespecified subgroups (age, gender, ethnicity, BMI, and type of surgery) by adding a treatment-by-subgroup interaction term to the binary regression models. *A priori*, we specified the expected direction of effects in each of the subgroups. Differences in general health status were compared between groups using Student's unpaired *t*-test.

We determined the association between patient characteristics and chronic postsurgical pain using exploratory logistic regression models. Factors that were found to be significant ($P<0.05$) in the univariate analyses were incorporated in the final multivariate logistic regression model.

A sensitivity analysis accounting for missing pain assessment at 1 yr after surgery was conducted using inverse probability weighting.[27] Briefly, the probability that a patient was successfully interviewed at 1 yr follow-up was estimated by logistic regression, adjusting for baseline characteristics. Outcome observations were then weighted by the inverse of these probability values to account for the possibility that those patients who responded to the pain assessment were not a true representative sample of the entire cohort. Patients who were successfully interviewed were therefore 'up-weighted' to represent themselves and patients similar to them who were not interviewed.

An exact test was used to verify the distribution of genotypes according to the Hardy–Weinberg equilibrium. The association between genotypes and chronic postsurgical pain was determined by a permutation procedure. Adjusted empirical family-wise probability values were calculated using PLINK-1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/download.shtml, last accessed 5 October, 2016).[28] All other analyses were performed using Stata version 12·1 (StataCorp, College Station, TX, USA). Reported *P*-values are two sided. Apart from the genetic association analysis, all statistical analyses were not adjusted for multiple comparisons. A *P*-value of <0.05 was considered statistically significant.

Ethical considerations

Ethics Committee approval was obtained from each of the participating centres, and all patients provided written informed consent.

Results

A total of 19 ENIGMA-II sites in five countries received ethics approval to conduct the chronic postsurgical pain study. Follow-up occurred between June 2010 and October 2014. Figure 1 shows the patient enrolment, treatment allocation, and follow-up in the study. Amongst 3518 eligible patients, 193 died before 12 month follow-up and were excluded from further analysis. Another 401 (12.1%) patients could not be contacted despite multiple attempts. The remaining 2924 patients were interviewed by telephone. Among these patients, 1446 were randomized to the nitrous oxide group and 1478 to the no nitrous oxide group. The median (interquartile range, IQR) time to follow-up was 400 (371–470) days.

Patient characteristics are summarized in Table 1. The mean age was 69.6 yr, with 35% of patients female and >60% classified as ASA III and IV. The commonest type of surgery was vascular surgery (32.6%), and 11.3% of patients had orthopaedic procedures. The median (IQR) duration of surgery was 2.7 (1.9–3.8) h.

At 12 month follow-up, 171 (11.8%) patients in the nitrous oxide group and 185 (12.5%) patients in the no nitrous oxide

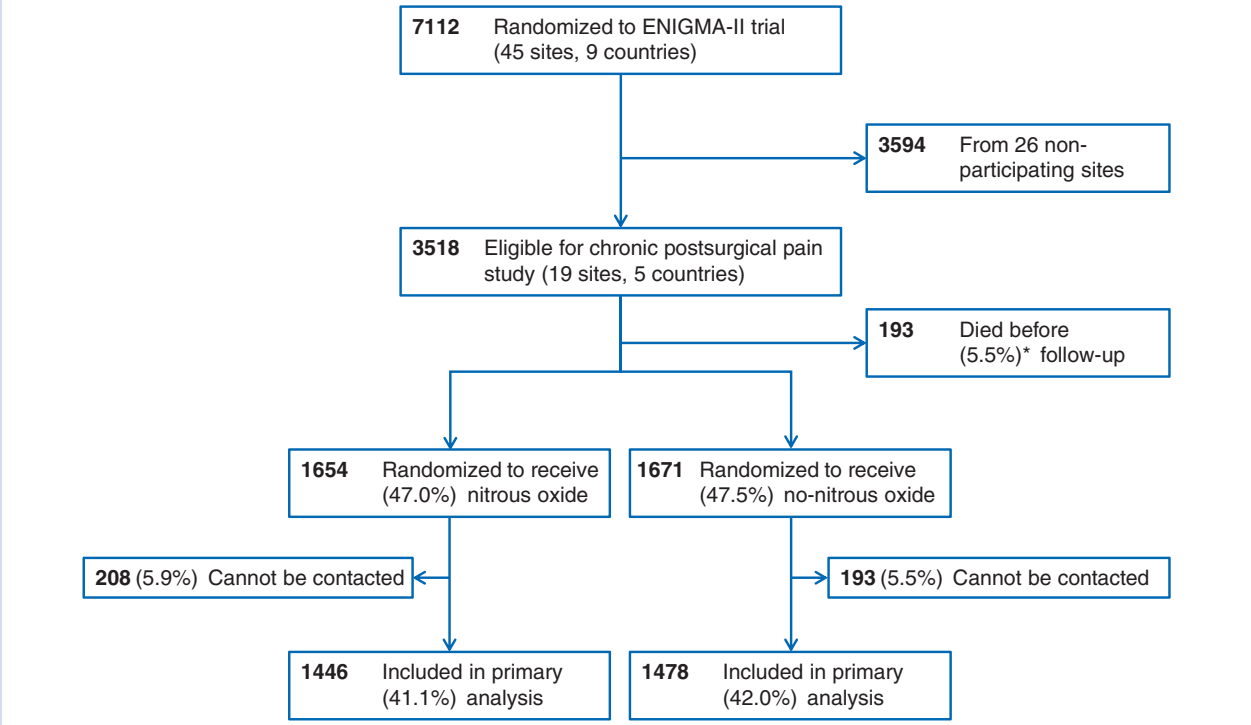

Fig 1 Trial flow chart. Values in parentheses indicate percentage of eligible patients for the chronic postsurgical pain study.

group reported chronic postsurgical pain. The relative risk (95% CI) for chronic postsurgical pain with nitrous oxide was 0.94 (0.75–1.17), P=0.57. The risk did not change significantly when adjusted for patients who were lost to follow-up, adjusted relative risk (95% CI): 0.92 (0.74–1.15), P=0.47.

Subgroup analyses for chronic postsurgical pain are shown in Fig. 2. Significant interaction was noted, suggesting that nitrous oxide reduced the risk of chronic postsurgical pain in Asian patients compared with non-Asian patients, relative risk (95% CI): 0.70 (0.50–0.98), P=0.031. The subgroup effect was consistent with our *a priori* hypothesis and remained significant when adjusted for missing data, adjusted relative risk (95% CI): 0.70 (0.50–0.97), P=0.048.

Among patients who reported chronic postsurgical pain, the median (IQR) worst, average, and least pain scores during the 24 h before telephone interview were 50 (20–70), 30 (10–50), and 10 (0–30) points, respectively. A total of 112 (3.8%) patients reported severe pain. The corresponding median (IQR) worst, average, and least pain scores were 70 (60–80), 55 (50–70), and 40 (20–50) points. Nitrous oxide did not impact on severe chronic postsurgical pain, relative risk (95% CI): 1.10 (0.76–1.61), P=0.62. In a subgroup analysis, two significant interactions were found (Supplementary Fig. S2). In patients with BMI >30 kg m^{-2} and patients having orthopaedic procedures, nitrous oxide appeared to increase the risk of severe chronic postsurgical pain. The interactions remained significant when adjusted for missing data.

Supplementary Table S1 summarizes the characteristics of chronic postsurgical pain. In patients reporting mild pain (pain score <50 points), the majority did not experience symptoms of neuropathic pain. In contrast, neuropathic pain was a prominent feature of severe chronic postsurgical pain. The impact of chronic postsurgical pain on daily activities is summarized in Supplementary Table S2. Patients with pain reported significant difficulties with general activity, mood, walking, relations with others, sleep, and enjoyment of life. Chronic pain also impaired mobility, self-care, and usual activity, in addition to provoking discomfort, anxiety, and depression (Supplementary Fig. S3, all P<0.001).

Table 2 shows the factors that were associated with the development of chronic postsurgical pain in the multivariate model. Younger age, female patients, prolonged surgery, and postoperative wound infection were independent risk factors for chronic postsurgical pain. Different types of surgery imposed variable risks. In general, patients having spinal and orthopaedic surgery were at higher risk. Adjustment for missing data did not substantially alter the risks for chronic postsurgical pain (Supplementary Table S3). Similar factors were noted to increase the risk of severe chronic postsurgical pain (Table 3 and Supplementary Table S4). Regional block and supplemental oxygen had no effect on the risk of development of chronic pain.

Blood samples were collected from 674 patients. Genotyping was successful in all patients for 1298A>C and in 98.9% of patients for 667C>T. The minor allele frequency was 39% for 1298A>C and 41% for 667C>T. Genotypic distributions were in Hardy–Weinberg equilibrium for both polymorphisms, with P-values of 0.053 and 0.078, respectively. Table 4 shows the association between genotypes and chronic postsurgical pain. Patients who were homozygous for either polymorphism were less likely to report chronic pain after surgery. Interestingly,

Table 1 Patient characteristics. Values are the number (percentage), mean (SD), or median (interquartile range, IQR). *Prior pain syndrome requiring regular analgesics

Characteristic	Nitrous oxide	No nitrous oxide	Lost to follow-up
Total number of patients	1486	1513	401
Age [yr; *n* (%)]			
<55	119 (8.2)	125 (8.5)	32 (8.0)
55–65	338 (23.4)	298 (20.2)	83 (20.7)
65–75	521 (36.0)	586 (39.6)	154 (38.4)
75-85	409 (28.3)	401 (27.1)	120 (29.9)
≥85	59 (4.1)	68 (4.6)	12 (3.0)
Weight [kg; mean (SD)]	76.7 (20.6)	77.2 (19.4)	–
BMI [kg m^{-2}; mean (SD)]	27.6 (6.4)	27.7 (6.1)	–
Female gender [*n* (%)]	509 (35.2)	534 (36.1)	156 (38.9)
Ethnicity [*n* (%)]			
Caucasian	922 (63.8)	979 (66.2)	–
Asian	469 (32.4)	440 (29.8)	–
Indian/Pakistan	13 (0.9)	15 (1.0)	–
Hispanic	5 (0.3)	5 (0.3)	–
Black	7 (0.5)	10 (0.7)	–
Other	30 (2.1)	29 (2.0)	–
ASA physical status [*n* (%)]			
I and II	510 (35.3)	518 (35.0)	71 (17.7)
III	845 (58.4)	857 (58.0)	287 (71.6)
IV and V	91 (6.3)	103 (7.0)	43 (10.7)
Medical history [*n* (%)]			
Hypertension	1218 (84.2)	1281 (86.7)	–
Coronary artery disease	491 (34.0)	500 (33.8)	–
Heart failure	79 (5.5)	97 (6.6)	–
Previous stroke	263 (18.2)	253 (17.1)	–
Current smoker	229 (15.8)	222 (15.0)	–
Diabetes mellitus	546 (37.8)	531 (35.9)	–
Prior history of pain syndrome*	45 (3.0)	42 (2.9)	–
Surgery type [*n* (%)]			
Colorectal	90 (6.2)	102 (6.9)	10 (2.5)
Ear, nose, throat, or maxillofacial	41 (2.8)	43 (2.9)	16 (4.0)
Gastrointestinal (non-colorectal)	176 (12.2)	165 (11.2)	45 (11.2)
Gynaecological	93 (6.4)	83 (5.6)	30 (7.5)
Hepatobiliary	112 (7.7)	104 (7.0)	37 (9.2)
Neurosurgery–spine	104 (7.2)	114 (7.7)	14 (3.5)
Orthopaedic	150 (10.4)	179 (12.1)	14 (3.5)
Plastics	22 (1.5)	14 (0.9)	6 (1.5)
Urology	149 (10.3)	173 (11.7)	38 (9.5)
Vascular	490 (33.9)	463 (31.3)	180 (44.9)
Other	19 (1.3)	38 (2.6)	11 (2.7)
Anaesthetic details			
Regional block [*n* (%)]	349 (24.1)	400 (27.1)	106 (26.4)
Duration of surgery [h; median (IQR)]	2.7 (1.9–3.8)	2.7 (1.9–3.7)	–
Duration of anaesthesia [h; median (IQR)]	3.2 (2.4–4.5)	3.2 (2.4–4.4)	–
Inspired oxygen concentration [%; median (IQR)]	30 (30–35)	33 (30–40)	–

significant benefit was observed only in patients randomized to nitrous oxide anaesthesia (interaction $P=0.047$ for 1298A>C and $P=0.042$ for 667C>T).

Discussion

In this 12 month follow-up study of 2924 ENIGMA-II trial participants, we found that chronic postsurgical pain was a common adverse event, affecting 12.2% of patients having major non-cardiac surgery. About one-third of the patients experienced severe pain with an average pain score ≥50 points in the previous 24 h before phone interview. Overall, nitrous oxide administration had no effect on the development of chronic pain, 12 months after index surgery. However, we observed a subgroup difference among Asian patients, suggesting a 30% decrease in the risk of chronic postsurgical pain with nitrous oxide. The latter finding is consistent with our previous trial, in which Asian patients benefited from nitrous oxide administration for the prevention of chronic postsurgical pain.[17]

The differential analgesic effect (among Asians *vs* non-Asians) may reflect genetic differences between ethnic populations.[29] In this respect, our data showed that patients

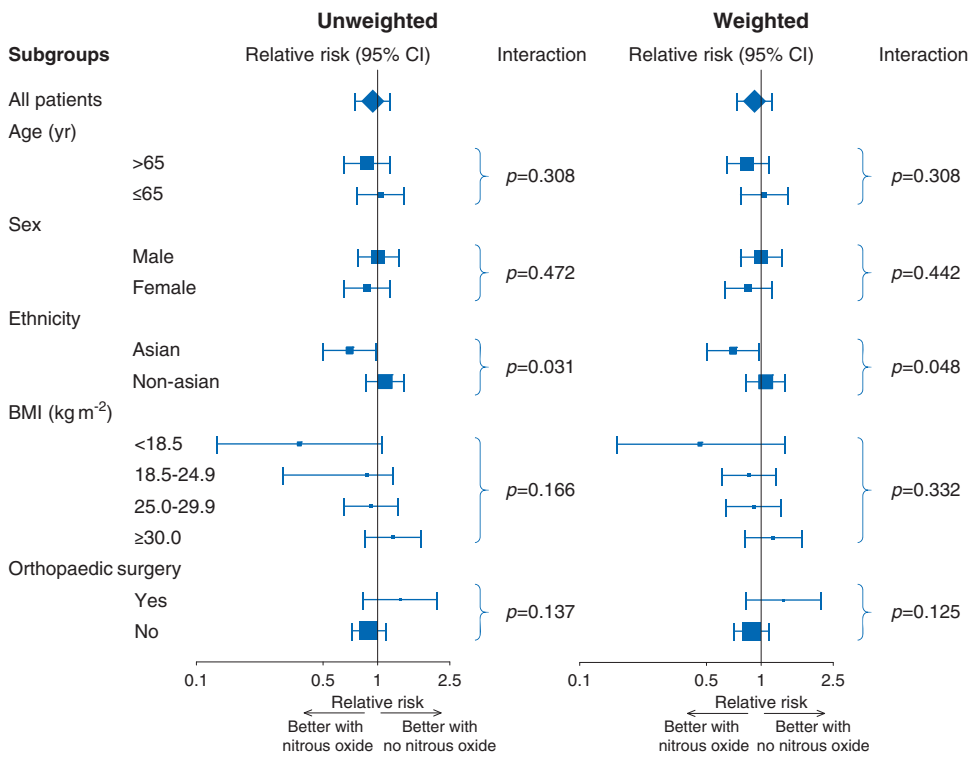

Fig 2 Relative risks for chronic postsurgical pain associated with the use of nitrous oxide in selected subgroups. The area of each square is proportional to the size of the corresponding subgroup. The horizontal bars indicate 95% confidence intervals (CIs).

carrying homozygous alleles of either 1298A>C or 667C>T polymorphism had a lower risk of chronic postsurgical pain when they received nitrous oxide. Interestingly, the allele frequencies in our Asian patients from Hong Kong were much higher than previously reported in Caucasians (39–41 *vs* 20–30%),[15 30] which might account for the better preventive analgesia with nitrous oxide in the Asian population. Unfortunately, we did not collect DNA samples from the non-Asian patients and cannot confirm this hypothesis in our trial. It remains plausible that long-term analgesia with nitrous oxide could be maximized in Caucasian patients with methylenetetrahydrofolate reductase gene polymorphisms. It should be noted that the majority of Asian patients in the ENIGMA-II trial were recruited from Hong Kong. Therefore, an unknown site effect, such as differences in postoperative pain management, may have influence the results.

In our subgroup analysis, we also observed an increased risk of severe chronic postsurgical pain with nitrous oxide in obese patients (with BMI >30 kg m^{-2}) and those having orthopaedic surgery, adjusted relative risks (95% CI): 2.26 (1.14–4.48) and 2.73 (1.15–6.46), respectively. It is unclear how nitrous oxide might predispose patients to the development of chronic pain. However, the subgroup effects were contrary to our *a priori* hypothesis and may represent random error. Great caution is required when interpreting these results.[31]

The mechanism underlying long-term analgesia with nitrous oxide has not been fully elucidated.[32] Evidence suggests that antagonism of excitatory amino acid neurotransmitters, such as those at the *N*-methyl-D-aspartate receptor, prevents the development of chronic pain.[9 10 33–36] Clearly, the protective effect of nitrous oxide, if it exits, outlasts intraoperative receptor antagonism, suggesting that other mechanisms may be involved. Transcriptional change in the spinal dorsal horn underpins the initiation of central sensitization and is a hallmark of chronic pain development.[37] In this regard, methionine synthase inhibition with nitrous oxide leads to a dose-dependent decrease in S-adenosyl methioine in the methylation cycle, resulting in DNA demethylation (Supplementary Fig. S1).[35] Given that methylation changes the binding capability of DNA to nuclear proteins and regulates transcription of nearby genes, it is plausible that nitrous oxide administration may modify initiation and maintenance of chronic pain.[38–40] A recent review suggests direct inhibition of methyltransferase, decreased DNA methylation, reduced thermal hyperalgesia, and reversed gene expression in a rat model of chronic inflammatory pain.[41] This epigenetic mechanism may also explain why nitrous oxide may not be suitable for the treatment of chronic pain, such as chronic low back pain,[42] a situation in which central sensitization has already been established.

Chronic postsurgical pain adversely affects daily activities. Consistent with previous reports,[47] our data demonstrated that it has a major impact on general health, affecting social function, emotion, and physical performance. Further study should focus on the economic consequences of chronic postsurgical pain, in terms of productivity, medical expenses, and costs of repeated hospital admissions.

Table 2 Factors predicting chronic postsurgical pain. CI, confidence interval

Characteristic	No pain	Chronic pain	Univariate model		Multivariate model	
	[*n* (%)]	[*n* (%)]	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
Age [per yr; mean (SD)]	69.6 (9.5)	67.3 (10.1)	0.98 (0.96–0.99)	<0.001	0.98 (0.96–0.99)	<0.001
BMI [per kg m^{-2}; mean (SD)]	27.6 (6.2)	27.9 (6.9)	1.01 (0.99–1.02)	0.474	–	–
Gender	–	–	–	0.003	–	<0.001
Male	1677 (65.3)	204 (57.3)	Reference		Reference	
Female	891 (34.7)	152 (42.7)	1.40 (1.12–1.76)		1.55 (1.21–1.97)	
Ethnicity				0.038		0.033
White	1690 (65.8)	211 (59.3)	Reference		Reference	
Asian	785 (30.6)	124 (34.8)	1.27 (1.00–1.60)		1.44 (1.08–1.92)	
Indian/Pakistan	22 (0.9)	6 (1.7)	2.18 (0.88–5.45)		2.24 (0.87–5.78)	
Black	13 (0.5)	4 (1.1)	2.46 (0.80–7.63)		2.54 (0.79–8.21)	
Other	48 (1.9)	11 (3.1)	1.84 (0.94–3.59)		1.52 (0.76–3.07)	
ASA physical status				0.703		
I and II	900 (35.0)	128 (36.0)	Reference			
III	1494 (58.2)	208 (58.4)	0.98 (0.77–1.24)		–	
IV and V	174 (6.8)	20 (5.6)	0.81 (0.49–1.33)		–	
Current smoker				0.059		
No	2184 (85.0)	289 (81.2)	Reference		–	
Yes	384 (15.0)	67 (18.8)	1.32 (0.99–1.76)		–	
Prior history of pain syndrome				0.035		0.229
No	2498 (97.3)	339 (95.2)	Reference		Reference	
Yes	70 (2.7)	17 (4.8)	1.79 (1.04–3.08)		1.41 (0.80–2.48)	
Surgery type				0.007		<0.001
Colorectal	171 (6.7)	21 (5.9)	1.20 (0.66–2.15)		1.11 (0.60–2.04)	
Ear, nose, throat, or maxillofacial	78 (3.0)	6 (1.7)	0.75 (0.30–1.86)		0.65 (0.26–1.65)	
Gastrointestinal (non-colorectal)	303 (11.8)	38 (10.7)	1.22 (0.74–2.02)		1.18 (0.70–1.98)	
Gynaecological	160 (6.2)	16 (4.5)	0.97 (0.51–1.84)		0.73 (0.37–1.42)	
Hepatobiliary	184 (7.2)	32 (9.0)	1.69 (1.00–2.88)		1.30 (0.75–2.25)	
Neurosurgery–spine	183 (7.1)	35 (9.8)	1.86 (1.11–3.14)		2.17 (1.27–3.73)	
Orthopaedic	272 (10.6)	57 (16.0)	2.04 (1.27–3.27)		2.87 (1.73–4.77)	
Plastics	31 (1.2)	5 (1.4)	1.57 (0.57–4.34)		1.85 (0.65–4.77)	
Urology	292 (11.4)	30 (8.4)	Reference		Reference	
Vascular	849 (33.1)	104 (29.2)	1.19 (0.78–1.83)		1.53 (0.98–2.38)	
Other	45 (1.8)	12 (3.4)	2.60 (1.24–5.44)		3.01 (1.40–6.50)	
Regional block				0.162		
No	1921 (74.8)	254 (71.3)	Reference		–	
Yes	647 (25.2)	102 (28.7)	1.19 (0.93–1.53)	–	–	–
Duration of surgery [per h; mean (SD)]	2.7 (1.7)	3.0 (2.2)	1.14 (1.07–1.21)	<0.001	1.16 (1.08–1.24)	<0.001
Inspired oxygen concentration [per %; mean (SD)]	31 (10.0)	31 (9.0)	1.00 (0.99–1.01)	0.970	–	–
Wound infection				<0.001		0.001
No	2371 (92.3)	308 (86.5)	Reference		Reference	
Yes	197 (7.7)	48 (13.5)	1.88 (1.34–2.63)		1.79 (1.26–2.56)	
Treatment allocation				0.568		
No nitrous oxide	1293 (50.4)	185 (52.0)	Reference		–	
Nitrous oxide	1275 (49.6)	171 (48.0)	0.94 (0.75–1.17)		–	

Although the ENIGMA-II trial randomized a large sample of patients, undergoing a wide range of non-cardiac surgery, to evaluate the effect of nitrous oxide on chronic postsurgical pain, our study has important limitations. We did not collect detailed baseline information about preoperative pain syndromes. Patients with pre-existing pain are thought to have a higher risk of developing chronic postoperative pain states.[3] However, inconsistent results have been reported in previous studies.[4344] Nevertheless, our follow-up questionnaires focused primarily on the presence of new-onset pain at the wound site 12 months after surgery, so that patients should have been able to distinguish such pain from other pre-existing pain conditions. We also did not record pain score or opioid use during the early postoperative period. Severe acute postoperative pain increases the risk of chronic postsurgical pain, and this may introduce bias.[7] However, our previous study showed that intraoperative nitrous oxide administration has no effect on early postoperative analgesia.[17] Finally, we did not adjust subgroup analyses for multiple testing, and this may increase type I error.

In summary, nitrous oxide administration during surgery did not reduce the risk of chronic postsurgical pain, in at-risk patients having major non-cardiac surgery. However, benefits may be possible in Asian patients and those carrying methylenetetrahydrofolate reductase gene polymorphisms. A more

Table 3 Factors predicting severe chronic postsurgical pain. CI, confidence interval

Characteristic	No pain	Chronic pain	Univariate model		Multivariate model	
	[n (%)]	[n (%)]	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P- value
Age [per yr; mean (SD)]	69.5 (9.6)	66.1 (9.9)	0.97 (0.95-0.98)	<0.001	0.96 (0.94-0.98)	<0.001
BMI [per kg m^{-2}; mean (SD)]	27.6 (6.2)	28.4 (7.2)	1.02 (0.99–1.05)	0.210	–	–
Gender				0.003		0.001
Male	1,677 (65.3)	57 (50.9)	Reference		Reference	
Female	891 (34.7)	55 (49.1)	1.78 (1.22–2.60)		1.93 (1.30–2.88)	
Ethnicity				0.070		
White	1690 (65.8)	74 (66.1)	Reference		–	
Asian	785 (30.6)	30 (26.8)	0.84 (0.55-1.30)		–	
Indian/Pakistan	22 (0.9)	2 (1.8)	1.90 (0.44–8.15)		–	
Black	13 (0.5)	3 (2.7)	5.29 (1.49–18.8)		–	
Other	48 (1.9)	3 (2.7)	1.32 (0.40–4.32)		–	
ASA physical status				0.316		
I and II	900 (35.0)	32 (28.6)	Reference			
III	1494 (58.2)	71 (63.4)	1.35 (0.89–2.07)		–	
IV and V	174 (6.8)	9 (8.0)	1.51 (0.71–3.22)		–	
Current smoker				0.129		
No	2184 (85.0)	89 (79.5)	Reference		–	
Yes	384 (15.0)	23 (20.5)	1.44 (0.90–2.30)		–	
Prior history of pain syndrome				0.011		0.088
No	2498 (97.3)	104 (92.9)	Reference		Reference	
Yes	70 (2.7)	8 (7.1)	2.66 (1.25–5.65)		1.97 (0.90–4.31)	
Surgery type				0.012		0.001
Colorectal	171 (6.7)	6 (5.4)	2.05 (0.82–15.2)		2.06 (0.61–7.00)	
Ear, nose, throat, or maxillofacial	78 (3.0)	2 (1.8)	1.55 (0.29–8.11)		1.39 (0.26–7.40)	
Gastrointestinal (non-colorectal)	303 (11.8)	12 (10.7)	2.31 (0.81–6.64)		2.43 (0.84–7.07)	
Gynaecological	160 (6.2)	16 (3.6)	1.47 (0.39–5.56)		0.94 (0.24–3.67)	
Hepatobiliary	184 (7.2)	9 (8.0)	2.76 (0.91–8.34)		2.14 (0.70–6.60)	
Neurosurgery–spine	183 (7.1)	15 (13.4)	4.68 (1.68–13.1)		5.31 (1.87–15.0)	
Orthopaedic	272 (10.6)	23 (20.5)	4.77 (1.79–12.7)		5.92 (2.15–16.3)	
Plastics	31 (1.2)	3 (2.7)	5.76 (1.32–25.2)		6.68 (1.47–30.3)	
Urology	292 (11.4)	5 (4.5)	Reference		Reference	
Vascular	849 (33.1)	30 (26.8)	2.06 (0.79–5.36)		2.30 (0.87–6.03)	
Other	45 (1.8)	3 (2.7)	3.52 (1.24–5.44)		3.58 (0.81–15.8)	
Regional block				0.465		
No	1921 (74.8)	80 (71.4)	Reference		–	
Yes	647 (25.2)	32 (28.6)	1.17 (0.77–1.78)		–	
Duration of surgery [per h; mean (SD)]	2.7 (1.7)	2.9 (2.1)	1.12 (1.01–1.24)	0.035	1.15 (1.03–1.29)	0.011
Inspired oxygen concentration [per %; mean (SD)]	31 (10.0)	32 (10.0)	1.00 (0.99–1.02)	0.636	–	
Wound infection				0.001		0.002
No	2371 (92.3)	93 (83.0)	Reference		Reference	
Yes	197 (7.7)	19 (23.2)	2.34 (1.40–3.90)		2.34 (1.36–4.02)	
Treatment allocation				0.615		
No nitrous oxide	1293 (50.4)	54 (48.2)	Reference		–	
Nitrous oxide	1275 (49.6)	58 (51.8)	1.10 (0.76–1.61)		–	

comprehensive pharmacogenetic study to explore this possibility is warranted.

Authors' contributions

Study design/planning: M.T.V.C., P.J.P., P.S.M., K.L.

Study conduct: M.T.V.C., P.J.P., P.S.M., S.W., K.L., N.B., M.J.P., W.S.B., D.I.S., Y.T., Y.C., W.K.K.W.

Data analysis: J.K., A.F., M.T.V.C., P.J.P.

Writing paper: M.T.V.C., P.J.P.

Revising paper: all authors.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

Declaration of interest

None declared.

Table 4 Association between methylenetetrahydrofolate reductase gene polymorphisms (1298A>C, 667C>T) and chronic postsurgical pain. CI, confidence interval

Genotypes	No pain (*n* (%)]	Chronic pain [*n* (%)]	Odds ratio (95% CI)	P-value
1298A>C				
All patients				
Wild-type	226 (86.3)	36 (13.7)	Reference	
Heterozygous	270 (91.5)	15 (8.5)	0.58 (0.34–1.03)	0.082
Homozygous	109 (94.8)	6 (5.2)	0.35 (0.14–0.85)	0.025
No nitrous oxide				
Wild-type	117 (85.4)	20 (14.6)	Reference	
Heterozygous	121 (87.7)	17 (12.3)	0.82 (0.41–1.65)	0.706
Homozygous	45 (91.8)	4 (8.2)	0.52 (0.17–1.61)	0.366
Nitrous oxide				
Wild-type	109 (87.2)	16 (12.8)	Reference	
Heterozygous	149 (94.9)	8 (5.1)	0.37 (0.15–0.89)	0.037
Homozygous	64 (97.0)	2 (3.0)	0.21 (0.05–0.96)	0.043
667C>T				
All patients				
Wild-type	187 (85.4)	32 (14.6)	Reference	
Heterozygous	313 (91.3)	30 (8.7)	0.56 (0.33–0.95)	0.043
Homozygous	101 (96.2)	4 (3.8)	0.23 (0.08–0.67)	0.007
No nitrous oxide				
Wild-type	95 (84.8)	17 (15.2)	Reference	
Heterozygous	136 (87.2)	20 (12.8)	0.82 (0.41–1.65)	0.121
Homozygous	53 94.6)	3 (5.4)	0.32 (0.09–1.13)	0.110
Nitrous oxide				
Wild-type	92 (86.0)	15 (14.0)	Reference	
Heterozygous	177 (94.7)	10 (5.3)	0.35 (0.150–0.80)	0.019
Homozygous	48 (98.0)	1 (2.0)	0.13 (0.016–0.99)	0.044

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Appendix

Evaluation of NitrousOxide in theGasMixture forAnaesthesia (ENIGMA)-IItrial

Steering Committee: P. Myles (Chair), K. Leslie, P. Peyton, M. Paech, P. J. Devereaux, D. Sessler, A. Forbes, S. Beattie

Data Safety Monitoring Board: H. Krum (clinical pharmacologist), J. Rigg (academic anaesthetist), D. McIlroy (academic anaesthetist), P. Ryan (independent statistician)

Endpoint adjudication committee: J. Tomlinson (internal medicine physician and anaesthetist), N. Badner (academic anaesthetist)

Data Quality Committee: P. Peyton (Chair), J. McNeil, A. Forbes, S. Wallace, A. Meehan, S. Poustie

Principal investigators and coordinators in the ENIGMA-II trial

*Member of Australian and New Zealand College of Anaesthetists Clinical Trials Network. †Sites participating in the Chronic Postsurgical Pain Long-Term Follow-Up Study

***Australia:**

†Alfred Hospital: P. Myles, S. Wallace, W. Galagher, C. Farrington, A. Ditoro
†Austin Hospital: P. Peyton, S. Baulch
Calvary Wakefield Hospital: D. Fahlbusch, D. Crump
†Dandenong Hospital: R. Bulach, D. Bryant
†Fremantle Hospital: E. O'Loughlin, V. Mitteregger
†Geelong Hospital: S. Bolsin, C. Osborne
†Monash Medical Centre: R. McRae, M. Backstrom
North West Regional Hospital: M. Reeves
†Peter MacCallum Cancer Centre: G. Thompson
†Royal Melbourne Hospital: K. Leslie, R. Cotter
†Royal Perth Hospital: M. Paech, S. March
Royal Prince Alfred Hospital: T. McCulloch
St Vincent's Hospital: B. Silbert, S. Said
†Westmead Hospital: R. Halliwell, J. Cope
Western Hospital: A. Jefferies

Canada

London Health Sciences Centre: N. Badner
†McMaster University: N. Buckley, T. Tidy
†Royal Victoria Hospital: T. Schricker, R. Latterman
†Toronto General Hospital: S. Beattie, J. Carroll
University of Alberta Hospital: M. Jacka, C. Bryden

Hong Kong

†Prince of Wales: *M. T. V. Chan, M. W. Y. Tsang
†Tuen Mun Hospital: B. C. P. Cheng, A. C. M. Fong
†Pamela Youde Nethersole Eastern Hospital: L. C. Y. Chu, E. G. Y. Koo

Malaysia

Hospital Kuala Lumpur: N. Mohd, L. E. Ming
Malaya Medical Centre: C. Wang

*New Zealand

†**Auckland Hospital:** D. Campbell, D. McAllister
Christchurch Hospital: R. Kennedy
†**Middlemore Hospital:** S. Walker, S. Olliff

Saudi Arabia

King Saud University Hospital: A. Eldawlatly, T. Alzahrani

Singapore

Tan Tock Seng Hospital: N. Chua

Switzerland

Geneva University Hospital: G. Haller, I. Pichon

UK

Blackpool Victoria Hospital: J. Cupitt
Bradford Teaching Hospital: A. Brennan
Hull Royal Infirmary: P. Balaji
King's College Hospital: G. Kunst
North Devon District Hospital: G. Rousseau
Plymouth NHS Trust: R. Sneyd, H. McMillan
Portsmouth Hospital: J. Nightingale
Royal Surrey County Hospital: M. Dickinson
University Hospitals, Coventry and Warwickshire: T. Saran
University Hospitals of Morecombe Bay, Lancaster: I. Parkinson

USA

Beth Israel Deaconess Medical Center: B. Subramaniam, V. Banner-Godspeed
†**Cleveland Clinic:** D. Sessler, A. Kurz, B. Hesler, J. Liu, A. Fu, C. Egan
Hillcrest Hospital: K. Cummings, N. Graham
Louisville Medical Centre: D. Obal, E. Cooke