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Associations Between Single-Nucleotide Polymorphisms and Epidural Ropivacaine Consumption in Patients Undergoing Breast Cancer Surgery

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Up to date, few published studies indicated the associations between genetic polymorphisms and epidural local anesthetics consumption. In this study, we investigated the associations between seven single-nucleotide polymorphisms (SNPs) and epidural ropivacaine consumption during breast cancer surgery in women from northeastern China. These seven SNPs (rs3803662 and rs12443621 in *TNCR9*, rs889312 in *MAP3K1*, rs3817198 in *LSP1*, rs13387042 at 2q35, rs13281615 at 8q24, and rs2046210 at 6q25.1) were identified by recent genome-wide association studies associated with tumor susceptibility. A total of 418 breast cancer women received thoracic epidural anesthesia with ropivacaine for elective mastectomy with axillary clearance. Their blood samples were genotyped for the seven SNPs using the SNaPshot method. For SNP rs13281615, the subjects with genotype AG and GG consumed a greater amount of the total epidural ropivacaine and the mean ropivacaine dose than the subjects with genotype AA ($p = 0.047$ and $p = 0.003$, respectively). Furthermore, no statistical differences were found in the total dose of ropivacaine, the mean consumption of ropivacaine, the onset of ropivacaine, or the initial dose of lidocaine among the three genotypic groups for the other six SNPs studied. Our study indicated that SNP rs13281615 at 8q24 was associated with the consumption of epidural ropivacaine during breast cancer surgery in northeastern Chinese women. It might provide new insights into the mechanisms of ropivacaine action and metabolism and facilitate the development of personalized medicine.

Introduction

Ropivacaine is a long-acting amide local anesthetic with a single enantiomer (S formation), whose action mechanism is similar to other local anesthetics. It blocks neuronal excitability and conduction through inhibiting neuronal sodium channels. In recent years, ropivacaine has been generally used in local infiltration, field block, intrathecal block, and other anesthesia methods because it has the characteristics of sensory and motor block differentiation and is less toxic for the central nervous system and the cardiovascular system (Hansen, 2004). The previous researches have shown that genetic susceptibility may have the relationship with the anesthetics, postoperative nausea and vomitting, and postoperative pain, such as μ receptor gene polymorphisms (*OPRM1*) and *CYP* gene polymorphisms, while whether there is a relationship between the genetic susceptibility and the dosage of ropivacaine still remains unknown (Wuttke *et al*., 2002; Kim *et al*., 2003; Klepstad *et al*., 2004; Candiotti *et al*., 2005; Chou *et al*., 2006a, 2006b; Han *et al*., 2006; Candiotti *et al*., 2009; Zhang *et al*., 2010, 2011). The epidural anesthesia with ropivacaine is widely used in the tumor surgeries. Recent genome-wide association studies (GWAS) identified seven novel single-nucleotide polymorphisms (SNPs) (rs3803662 and rs12443621 in *TNCR9*, rs889312 in *MAP3K1*, rs3817198 in *LSP1*, rs13387042 at 2q35, rs13281615 at 8q24, and rs2046210 at 6q25.1) (Easton *et al*., 2007; Stacey *et al*., 2007; Zheng *et al*., 2009) for breast cancer with high frequency. When we assessed the associations of these seven SNPs with breast cancer risk in a northern Chinese Han population, we also evaluated the associations of these undefinite functional SNPs with ropivacaine dose and efficacy. It might provide the rationale for personalized medicine of local anesthetics.

Therefore, in this study, we chose these seven SNPs (rs3803662, rs12443621, rs889312, rs3817198, rs13387042, rs13281615, and rs2046210), relating with tumor susceptibility identified by GWAS, and evaluated the associations between these SNPs and the intraoperative consumption of epidural ropivacaine among the northern Han women in China.

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Methods

Patients

With the approval of the ethics committee of the Harbin Medical University, and after obtaining written informed consent, we recruited into the study all American Society of Anesthesiologists physical status I or II, Chinese Han women with breast cancer who were diagnosed by preoperative puncture pathological results and scheduled to have an elective mastectomy with an axillary clearance at the Department of Breast Surgery of the Third Affiliated Hospital of Harbin Medical University in the 7-month period from November 1, 2008, to May 31, 2009. Patients were excluded from the study if they underwent immediate breast reconstruction, breast-conserving therapy, or bilateral breast surgery or if they had any contraindications for thoracic epidural anesthesia (TEA), infection at the site of the planned epidural placement, any coagulation disorders, or any known allergies to ropivacaine or other local anesthetic agents. These patients were not genetically related within three generations. A total of 418 Chinese women with breast cancer were recruited for this study.

Anesthetic procedure

Basic vital signs, including the heart rate, noninvasive blood pressure, pulse oximetry, and respiratory rate were monitored before the induction of anesthesia. Intravenous access was established before anesthesia, and 5 mL of venous blood was collected for the preparation of DNA specimens. All the patients were given 500 mL of the lactated Ringer's solution for prehydration before anesthesia. Mild sedation was intravenously induced using 1–2 mg midazolam or 50–100 μg fentanyl as needed for TEA. TEA was performed with the patient in the right lateral position, and a 16-gauge Tuohy needle was inserted at the T3–4 level. The thoracic epidural space was confirmed by loss of resistance. An epidural catheter was measured and inserted 3–5 cm into the epidural space through the Tuohy needle. A test dose of 3–5 mL of 1.33% lidocaine with 1:200,000 epinephrine was delivered with repeated aspiration guided by the sensory effect to exclude intravascular or intrathecal injection. After the insertion of the catheter, the patient lay in the supine position. The patients and attending anesthesiologists were blinded to the patient's genotype during the surgery. An initial titrated dose of 5–10 mL of 0.375% ropivacaine was injected through the catheter. The operation began when the sensory block to cold was detected at the T2 level of the patient's midline. In cases that the sensory block was not adequate, an additional dose of 3–5 mL of ropivacaine was individually titrated. Supplemental oxygen (3–6 L/min) was administered via a face mask during the surgery. Patients with completely unsuccessful blocks were given general anesthesia and excluded from this study. The catheter was removed immediately at the end of the surgery.

During the operation, hypotension and hypertension (±20% deviation from baseline), bradycardia (heart rate <50 bpm), and tachycardia (heart rate >100 bpm) were recorded. Hypotension was treated with a bolus of ephedrine and intravenous crystalloids. Hypertension was treated by increasing the depth of the anesthesia. Antiemetics (intravenous 8 mg ondansetron) and a nonsteroidal anti-inflammatory agent (intravenous 1 mg/kg flurbiprofen axetil) were routinely administered before the end of the operation. Oral analgesic agents (diclofenac sodium and codeine phosphate tablets/metamorphine, total dose of 240 mg/24 h) were administered if the patient reported pain at a visual analogue scale value of more than 4 during the first 24 postoperative hours. The patient's age, height, weight, body mass index (BMI), the initial dose of lidocaine, the total consumption of intraoperative ropivacaine, the onset of ropivacaine, and the duration of surgery were recorded. The mean dose of intraoperative ropivacaine used was then calculated.

Genotyping

Genomic DNA was isolated from EDTA anticoagulated whole blood using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biotechnology). The SNaPshot SNP assay was performed to detect dimorphisms at the seven SNPs (Table 1). The resulting data were analyzed using the GeneMapper™ 4.0 Software (Applied Biosystems). For quality control, the genotyping was performed without the knowledge of the subjects' status, and 5% of the cases were

Table 1. Primers Used for Genotyping the Seven Single-Nucleotide Polymorphisms

Target SNP	*Region*	*Sequence (5′ > 3′)*
rs3803662	16q12.1/*TNCR9*[a]	Forward:5′-TCATCCAAAGCACCAACTATGAGAGA-3′ Reverse:5′-CAAAGACCACCGGCTGAACAA-3′
rs12443621	16q12.1/*TNCR9*[a]	Forward:5′-GACCACTGCAGAAAAGGGAGAGC-3′ Reverse:5′-TGGAGCCTAGTAAGCCAGGATTTGA-3′
rs889312	5q11.2/*MAP3K1*	F:5′-CTGAGATGCCCCTGCTGGAGA-3′ Reverse:5′-TGCCCTGAAGTGAGTAGGGCTGT-3′
rs3817198	11p15.5/*LSP1*	Forward:5′-CAAGGGTGGACTCCGAGTTGG-3′ Reverse:5′-CTGCTGTGGCTTTTGGGGAGT-3′
rs13387042	2q35/unknown	Forward:5′-AAACCAGAACAGAAAGAAGGCAAATG-3′ Reverse:5′-GATCCACTAGTGTGGTGAAGGAAGA-3′
rs13281615	8q24.21/unknown	Forward:5′-tggggaaaaGTTCATGAATTCCGTA-3′ Reverse:5′-TCTCCCCCAAACCCCCTACTC-3′
rs2046210	6q25.1/unknown	Forward:5′-GAAACCATCAGGGTGCCTCAA-3′ Reverse:5′-TCATAGCATTCAGCTTCCCAATGA-3′

[a]Also known as *TOX3*, as reported initially.
SNP, single-nucleotide polymorphisms.

randomly selected for replicate genotyping by a different technician; the reproducibility was 100%. Genotyping failed in only one case due to low DNA quality, and the average call rate for all SNPs was higher than 99%.

Statistical analyses

Numerical variables were estimated for normality using the Shapiro–Wilk test in three genotypic groups for each of the seven SNPs. Any variables that satisfied this assumption were expressed as the mean and SD; if the variables did not satisfy this assumption, they were expressed as the median [interquartile range]. The differences for the observed variables among the three genotypic groups of each SNP were assessed using one-way analysis of variance for variables that satisfied normality or, by the Kruskal–Wallis tests for variables with skewed distributions. Homozygotes for the nonrisk allele were used as the reference group, and the dominant model was analyzed using the homozygotes for the risk allele and heterozygotes versus the reference group by the Student's *t*-test or the Mann–Whitney *U* test. The Hardy–Weinberg equilibrium (HWE) was tested among the genotypes for each SNP. This sample size had a statistical power higher than 90% under the dominant model for the seven SNPs studied. Except for rs3817198 and rs13387042, this sample size had a statistical power higher than 80% under the codominant model for other five SNPs. In this study, because the number of subjects carrying only the risk allele of rs3817198 and rs13387042 was less than five, the statistical power was less than 50% when the genotype was compared between the homozygotes for the nonrisk allele and the homozygotes for the risk allele for SNP rs3817198 and rs13387042, respectively. All statistical tests were two sided, and a p-value < 0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS for Windows (version 13.0; SPSS). Power analyses were performed using G*Power v.3.1.5 (Faul *et al*., 2007).

Results

A total of 417 patients completed the study except for 1 patient, and their clinical data are summarized in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/gtmb). The average patient age was 49.4 years (range 27–80), and the mean BMI was 24.3 (range 15.8–34.8). The patients underwent unilateral modified radical mastectomy with some degree of axillary node dissection. There were no failures in the placement of the epidural catheter. All surgeries were randomly performed by Professor D.P. or Professor Z.L. or Professor G.Z. Hypotension, hypertension, tachycardia, and bradycardia were the common mild side effects ($\leq 10\%$ of the baseline) in all three genotypic groups for all seven SNPs during the entire perioperative period. The estimated blood loss was minimal and similar in all three genotypic groups for all seven SNPs; additionally, none of the patients required blood transfusion and rescue analgesics. The genotype distributions of the seven SNPs are displayed in Table 2. These genotype frequencies were in HWE.

There were no significant differences in the age and BMI among the three genotypic groups for each of the seven SNPs (seen in the Supplementary Table S1). For SNP rs13281615 at 8q24, it was found that the subjects with the genotype AG and GG consumed a greater amount of the total epidural ropivacaine than the subjects with the genotype AA ($p = 0.047$) (Table 3). The mean consumption of epidural ropivacaine was also significantly greater in patients with the genotype AG and GG of SNP rs13281615 than those with the genotype AA. However, there were no statistical differences in the onset of ropivacaine, the initial dose of lidocaine, and the duration of surgery between patients with the genotype AG and GG and those with the genotype AA.

Table 2. Genotype Distribution for the Seven Screened Single-Nucleotide Polymorphisms

rs3803662	GG:45(10.8%)/GA:170(40.8%)/AA:202(48.4%)
rs12443621	GG:146(35.0%)/GA:196(47.0%)/AA:75(18.0%)
rs889312	AA:112(26.9%)/AC:191(45.8%)/CC:114(27.3%)
rs3817198	TT:311(74.6%)/TC:102(24.5%)/CC:4(0.9%)
rs13387042	GG:319(76.5%)/GA:95(22.8%)/AA:3(0.7%)
rs13281615	AA:100(24.0%)/AG:215(51.5%)/GG:102(24.5%)
rs2046210	GG:139(33.3%)/GA:203(48.7%)/AA:75(18.0%)

Data are expressed as the number (%) of subjects.

For the SNP rs889312, the duration of surgery was significantly shorter in patients with the genotype AC and CC than in those with the genotype AA. However, no significant differences were found in the initial dose of lidocaine, the onset of epidural ropivacaine, the total consumption of epidural ropivacaine, and the mean consumption of epidural ropivacaine in patients with the genotype AC and CC compared to those with the genotype AA.

Moreover, there were no significant differences in the initial dose of lidocaine, the onset of epidural ropivacaine, the total consumption of ropivacaine, the mean consumption of epidural ropivacaine, and the duration of surgery between patients with the heterozygote and homozygote for the risk allele and those with the heterozygote for the nonrisk allele for other five SNPs.

Discussion

Our major finding was that the SNP rs13281615 at 8q24 was associated with a significant variability in the intraoperative epidural ropivacaine dose during mastectomy with axillary clearance among women from the Heilongjiang Province in northeastern China. In 2007, rs13281615 at 8q24 was identified through GWAS by Easton to increase breast cancer risk in European individuals (Easton *et al*., 2007). Additionally, previous studies suggested that many variants at 8q24 increased prostate (Haiman *et al*., 2007b; Yeager *et al*., 2007) and colorectal (Gruber *et al*., 2007; Haiman *et al*., 2007a; Tomlinson *et al*., 2007; Zanke *et al*., 2007) cancer risk. To date, no functional genes have been reported at 8q24. In our study, ropivacaine was used due to its more rapid onset, its longer duration of analgesia, and its larger initial spread (Hura *et al*., 2006). Ropivacaine was also described as being less potent, less cardiotoxic, and less neurotoxic than bupivacaine (Akerman *et al*., 1988; Feldman and Covino, 1988; Concepcion *et al*., 1990; Whitehead *et al*., 1990). However, up to date, a few published studies suggested the associations between genetic polymorphisms and the consumption of epidural ropivacaine or other local anesthetics.

For rs13281615, the global minor allele frequency (MAF) G in PUBMED was 0.482, its frequency in European population was 0.40, its frequency in the southern Chinese people was 0.515, and its frequency in our observed population was 0.502.

Table 3. Patients' Demographic and Clinical Data

	rs13281615					
	AA	*AG*	*GG*	*AG+GG*	p^a	p^b
N	100	215	102	317		
Age, year	49.1±10.9	49.0±9.7	50.3±10.6	49.4±10.0	0.526	0.764
Weight, kg	62.2±9.7	61.9±9.0	64.5±10.3	62.7±9.5	0.060	0.590
Height, cm	160.7±4.6	160.1±4.5	161.3±4.6	160.5±4.6	0.070	0.666
BMI, kg/m^2	24.0±3.4	24.2±3.4	24.8±3.4	24.4±3.4	0.253	0.418
Initial lidocaine, mg	66.5 [66.5,66.5]	66.5 [66.5,66.5]	66.5 [66.5,66.5]	66.5 [66.5,66.5]	0.806	0.584
Total ropivacaine, mg	50.8 [38.1,67.9]	52.6 [40.6,67.8]	61.4 [50.0,75.7]	54.5 [42.0,71.5]	0.001	0.047
Onset of ropivacaine, min	6.0 [5.0,7.0]	6.0 [5.0,7.0]	6.0 [5.0,8.0]	6.0 [5.0,7.0]	0.103	0.055
Duration of surgery, min	100.0 [75.0,120.0]	90.0 [75.0,120.0]	105.0 [80.0,120.0]	90.0 [75.0,120.0]	0.163	0.573
Mean ropivacaine, mg/min	0.5 [0.4,0.7]	0.6 [0.4,0.8]	0.6 [0.5,0.8]	0.6 [0.5,0.8]	0.003	0.003

Data are expressed as numbers, mean±SD or median [interquartile range].
[a]*p*-Value comparing the three genotype groups separately.
[b]*p*-Value in a dominant model, comparing the group of homozygotes for the nonrisk allele with the combined group of heterozygotes and homozygotes for the risk allele.
BMI, body mass index.

Our study suggested that subjects homozygous for the G risk allele consumed the most epidural ropivacaine (i.e., they displayed a greater mean consumption of intraoperative ropivacaine than patients with other genotypes). Therefore, we speculated that it could be associated with a lower sensitivity of the patients with the genotype GG to the anesthetic effect of epidural ropivacaine that increased the dose of a local anesthetic, or a lower pain threshold in counteracting intraoperative pain for patients with the genotype GG, or a combined effect. Furthermore, we observed a dose-dependent effect of the G risk allele; each additional copy increased the total consumption of ropivacaine.

The characteristics of GWAS were to find some unknown genes or chromosome domains, and provided us some clues to understand the mechanisms of complicated human diseases. Although GWAS could identify a mount of genetic loci or domains, few had the definite biological functions. The seven SNPs studied in our research were identified by recent three-stage GWAS associated with tumor susceptibility with high frequencies, and most of them were without definite biological functions. More and more studies have shown that the efficacy and reaction of the same anesthetic differ in different populations and individuals due to genetic polymorphisms. The genetic variations of drug-metabolizing enzymes, transporters, and receptors (targets of drug action) were the main reasons causing individual differences in the drug response, and these also influenced the anesthetic drug uses. Most of these genetic variations were identified by association studies based on the candidate-gene strategy, and few were identified by GWAS to be about drug reactions. It was rare to report the associations between an indefinite functional SNP identified by GWAS and the dosages of anesthetics. Epidural anesthesia with local anesthetics is widely used in tumor surgery. When we assessed the associations of SNPs identified by GWAS with tumor susceptibility in a northern Chinese Han population, we also evaluated the associations of these SNPs with a local anesthetic dose and efficacy. It is helpful to discover new SNPs and genes associated with pharmacogenetics, to promote pharmacokinetics development, to facilitate the process of personalized medicine, to advance the development of individual medicine. Ropivacaine is a well-tolerated regional anesthetic effective for surgical anesthesia as well as the relief of postoperative and labor pain. In our study, we aimed to evaluate the associations between these seven SNPs without definite gene functions and the intraoperative consumption of epidural rapivacaine among the northern Han women in China. Our results showed that some SNPs were not only associated with breast cancer risk (Jiang *et al.*, 2011), but also associated with the intraoperative epidural ropivacaine dose among the northern Han women in China. Our studies suggested that there was a possibility that a functional gene would exist at the 8q24 domain, relating to ropivacaine of drug-metabolizing enzymes, transporters, receptors (targets of drug action), etc. The frequency of genetic variations varies in different ethnic populations, even as there is genetic variation between the southern and northern people in China (Jakobsson *et al.*, 2008; Xu *et al.*, 2009). Therefore, whether this influence exists in other ethnic populations remains to be proven in further research.

In conclusion, our studies evaluated the associations of the seven SNPs with the intraoperative consumption of epidural ropivacaine in Chinese Northern Han women, and found that the individuals with the G allele of rs13281615 at 8q24 required more epidural ropivacaine. This study might contribute to the development of ropivacaine pharmacokinetics and provide new insights for the personalized medicine of local anesthetics. Further studies are needed to characterize the functional sequences that cause individual differences of epidural ropivacaine consumption.

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Author Disclosure Statement

No competing financial interests exist.

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