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Catechol-O-methyltransferase polymorphisms do not play a significant role in pain perception in male Chinese Han population

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Xiang X, Jiang Y, Ni Y, Fan M, Shen F, Wang X, Han J, Cui C. Catechol-O-methyltransferase polymorphisms do not play a significant role in pain perception in male Chinese Han population. *Physiol Genomics* 44: 318–328, 2012. First published January 17, 2012; doi:10.1152/physiolgenomics.00162.2011.—Polymorphisms in the human catechol-O-methyltransferase (COMT) gene have been widely studied for their role in pain and analgesia. In this study, sensitivity to potassium iontophoresis, visual analog scale measurements for fixed twofold pain threshold stimulation and pain threshold changes induced by transcutaneous electrical acupoint stimulation (TEAS) were assessed in a population of healthy Chinese males. These results were correlated with the alleles of six single nucleotide polymorphisms (SNP) or diplotypes of common haplotypes designated as low pain sensitive, average pain sensitive, and high pain sensitive in the COMT gene of these subjects. Our results reveal that the alleles of each SNP are not significantly correlated with pain perception except for the rs4633 allele in the 2 Hz TEAS session ($P < 0.05$). In addition, the six diplotypes of COMT haplotypes, which cover 92.5% of the Chinese population, are also not correlated with pain perception. Moreover, there were no significant differences in pain threshold changes induced by 2 and 100 Hz TEAS among the diplotypes of each SNP or the various haplotypes. These results suggest that COMT activity do not play a significant role in pain perception and TEAS-induced analgesia in the Chinese Han male population.

gene polymorphism; haplotype; analgesia; electroacupuncture

CATECHOL-O-METHYLTRANSFERASE (COMT) is a pivotal regulator of brain dopamine function that has a region-specific role (17) and has recently been implicated in the regulation of pain perception (7, 8). Association studies examining COMT gene polymorphisms and pain perception have generated conflicting results. One functional polymorphism at gene codon 158, rs4680, results in a valine to methionine substitution in the COMT (21) and a 65–75% reduction in enzyme activity (23). In addition, myofacial pain patients have been shown to exhibit lower COMT activity relative to controls (24). Low COMT activity has been associated with heightened pain perception in humans and increased experimental pain sensitivity in rodents via stimulation of the β_2- and β_3-adrenergic receptors (26). In a recent study, four single nucleotide polymorphisms (SNPs) located in the central region of the COMT gene (rs6269, rs4633, rs4818, and rs4680) have been shown to build up a haplotype. Three major COMT haplotypes, GCGG, ATCA, and ACCG, have been designated as low pain sensitive (LPS), average pain sensitive (APS), and high pain sensitive haplotype (HPS), respectively, and were strongly associated with human pain perception (7, 8). In contrast, Kim and colleagues (20) did not find an association between rs4680 and variations in heat and cold pain sensitivity in humans. It is known that population stratification can cause false associations to be made because of the pattern of genetic variations and inconsistent pain ratings that occur in various ethnic populations (16, 19). Another issue to be considered when analyzing mixed ethnic samples is that the haplotype frequencies and block structures may differ among different ethnic groups (29).

In China, acupuncture has been used to treat highly diversified pain for thousands of years, and it has more recently been recognized (at a milestone National Institutes of Health consensus conference in 1997) in the West as a useful complementary medicinal approach. The analgesic potency and the underlying neurobiological mechanisms of electroacupuncture (EA) and transcutaneous electrical acupoint stimulation (TEAS), in which the acupuncture needles and skin electrodes are placed at the same acupoints, are thought to be very similar, if not identical (13, 38). Our previous studies have demonstrated that different frequencies of TEAS can induce analgesia by mobilizing the release of specific neuropeptides in the central nervous system. Peripheral stimulation of 2 Hz produces a significant increase in the content of enkephalin immunoreactivity (IR) but not in that of dynorphin IR, whereas 100 Hz peripheral stimulation increases dynorphin IR but not enkephalin IR (13). The central pathways that mediate low and high frequency EA analgesia have also been explored. Signals from 2 Hz EA seem to sequentially activate the arcuate nucleus of the hypothalamus (β-endorphinergic neurons), periaqueductal grey (PAG), medulla (enkephalinergic neurons), and the dorsal horn to suppress nociceptive transmission, 100 Hz EA has been shown to activate a short parabrachial nucleus-PAG-medulla-spinal dorsal horn pathway that involves dynorphin (15). Thus, growing evidence suggests that 2 and 100 Hz EA represent two distinct therapeutic entities (12). Previous studies have shown that droperidol, a dopamine (DA) receptor antagonist, can potentiate EA-induced analgesia by antagonizing the activity of the dopaminergic system (42). Low COMT activity has been reported to increase pain sensitivity and to decrease the activation of brain μ-opioid system after an experimental pain challenge (43). In addition, low COMT activity (Met/Met) individuals have also been shown to exhibit reductions in the neuronal content of enkephalin peptides and an increased in regional μ-opioid receptor levels. In contrast, reductions in

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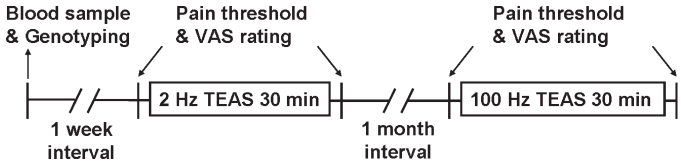

Fig. 1. Chronological order of the experiments.

dopaminergic neurotransmission [as observed in high COMT activity (Val/Val) individuals] result in opposite effects on the μ-opioid system (1).

COMT inhibitors have been proven to have antihyperalgesic effect; they even have been proven to be a potential analgesic drug (30). Here, we investigated the potential associations between alleles of COMT SNPs or diplotypes of haplotypes and the pain induced by potassium iontophoresis in young Chinese men. In addition, we investigated the relationships between TEAS analgesia and the COMT polymorphisms in this population.

MATERIALS AND METHODS

Participants. A total of 200 healthy Chinese Han males, aged 18–30 yr old, were recruited from blood donors in Beijing, China. Male were exclusively recruited for this study to avoid any possible effects produced by female menstrual cycles. In compliance with the guidelines of human experiments from the local ethical committee of Peking University, each subject had a sufficient understanding of the purpose and procedure used in the study and provided his informed consent. In addition, all subjects were free to withdraw from the experiment at any time. None of the subjects had a previous history of psychiatric or neurological disorders, and the subjects did not exhibit any pain or distress at the time of the study. Only one subject had any prior experience with acupuncture therapy, and we were unable to follow up with two of the participants in this study.

Pain measurements. The device used to measure pain threshold is controlled from a single-chip computer that is powered by a constant-current power source. The cathode consists of a copper plate (8 × 8 cm) that is covered with several layers of saline-saturated medical gauze, which was placed on the surface of the subject's leg. The anode consists of a piece of wet cotton (with a diameter of 5 mm) that had been previously soaked with KCl solution and was placed on the volar surface of the subject's arm. The potassium ions are carried by the gradually increasing anode current (at an increasing speed of 0.1 mA/s) into the skin to produce acute chemical pain (18). The current was terminated immediately when the subject reported that the stimulus was painful (39). The average pain threshold was determined from a total of five consecutively performed tests that had an intertest interval of 1 min. The standard pain stimulus given to each subject was double the intensity of his basal pain threshold. This twofold pain threshold stimulus was applied for 30 s before and after TEAS

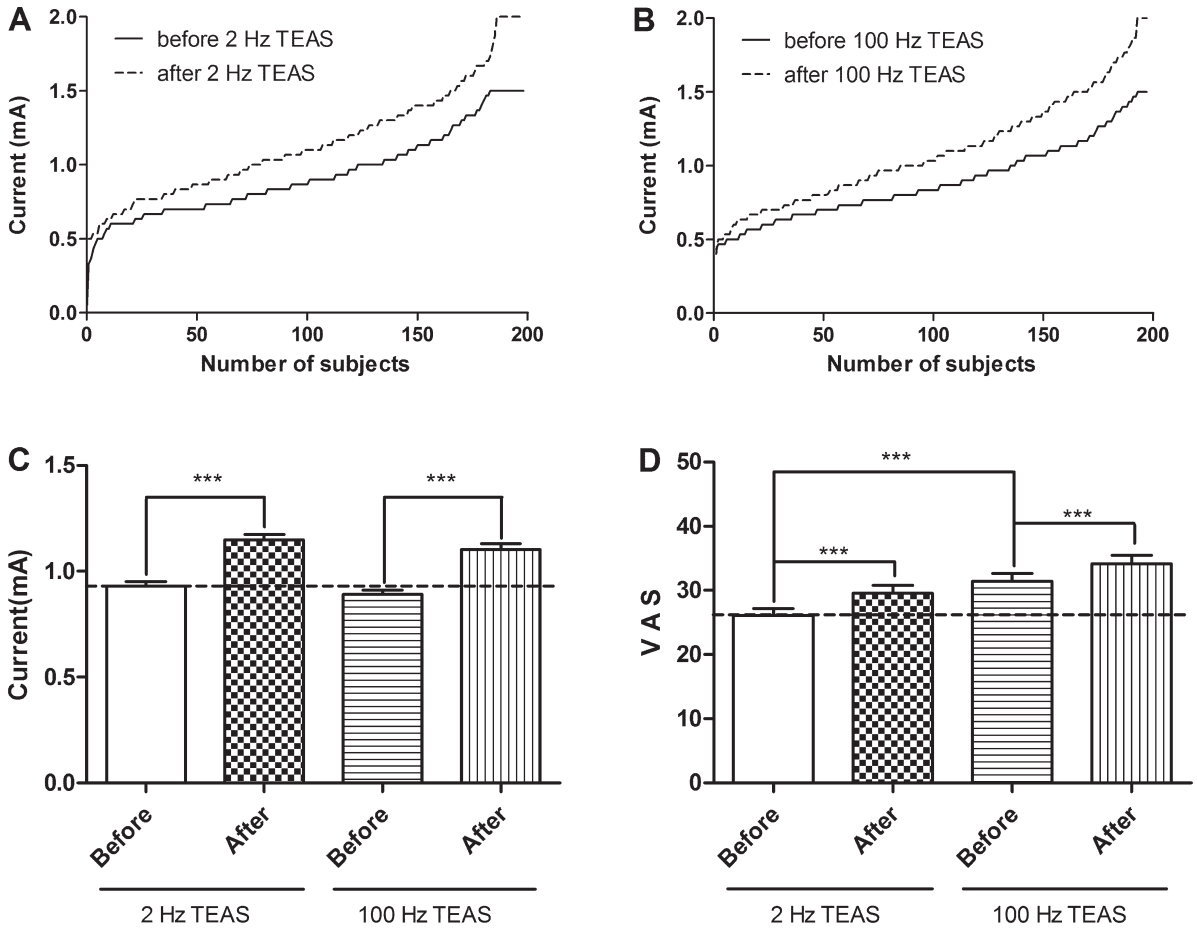

Fig. 2. Pain threshold and visual analog scales (VAS) of the fixed 2-fold pain threshold stimulation changed significantly after 2 or 100 Hz TEAS. The distributions of the pain threshold for each individual (*A, B*) are arranged from the lowest to highest intensity (the *x*-axis represent the accumulative number of subjects). The pain threshold increased significantly after 2 Hz TEAS (*A, C*) or 100 Hz TEAS (*B, C*). The VAS of the fixed 2-fold of pain threshold stimulation increased significantly depending on the sequence of the test. ***$P < 0.001$.

Table 1. *MAF of the six SNPs in Chinese males participating in this study compared with the HapMap database records*

SNP Loci	Minor Allele	MAF, %	
		Our Results	HapMap
rs2075507	G	24.8	
rs6269	G	40.2	
rs4633	T	24.8	23.3
rs4818	G	39.8	38.9
rs4680	A	26.5	25.6
rs165599	G	46.8	

SNP, single nucleotide polymorphism; MAF, minor allele frequency. The HapMap database is at http://hapmap.ncbi.nlm.nih.gov/.

respectively, which was usually in the range of 1.8–2.0 mA and applied to the mirror place on the other forearm. The subjects were then asked to rate the pain on a 100-mm visual analog scale (VAS), where 0 mm represented no pain and 100 mm was considered to be the worst possible pain. The chronological order of the experiments performed is depicted in Fig. 1.

Transcutaneous electrical acupoint stimulation. The transcutaneous electrical nerve stimulation (TENS) device (HANS-200E; Nanjing Gensun Medical Technology, Nanjing, China) is a battery-driven dual-channel acupoint nerve stimulator with two pairs of constant current electric outputs that send electrical impulses through two adhesive electrode-pads that are placed on the skin. When the electrode pads are placed at acupoints, TENS is referred to as TEAS. Hegu (LI4, located at the midpoint of the second metacarpus on the radial side) and Laogong (PC8, midway between the second and third metacarpus on the palmar side, where the middle finger falls when the hand makes a fist) (6) of the same hand, which together form an electric circuit, are two acupoints commonly used for TEAS (5, 14). The advantages of TEAS over EA are its noninvasiveness and the ease of its application. The frequency of the output stimulation can be 2 Hz (0.6-ms pulse width) or 100 Hz (0.2-ms pulse width). We measured the analgesic effects induced by TEAS in 2 and 100 Hz sessions in all subjects. Before and after the 30 min TEAS in each session, pain thresholds and VAS ratings were measured as previously described. The interval between the two sessions was at least 1 mo to eliminate any potential influences of the first session on the second session. The pain threshold measurements taken prior to TEAS in each session were used as the basal pain perception.

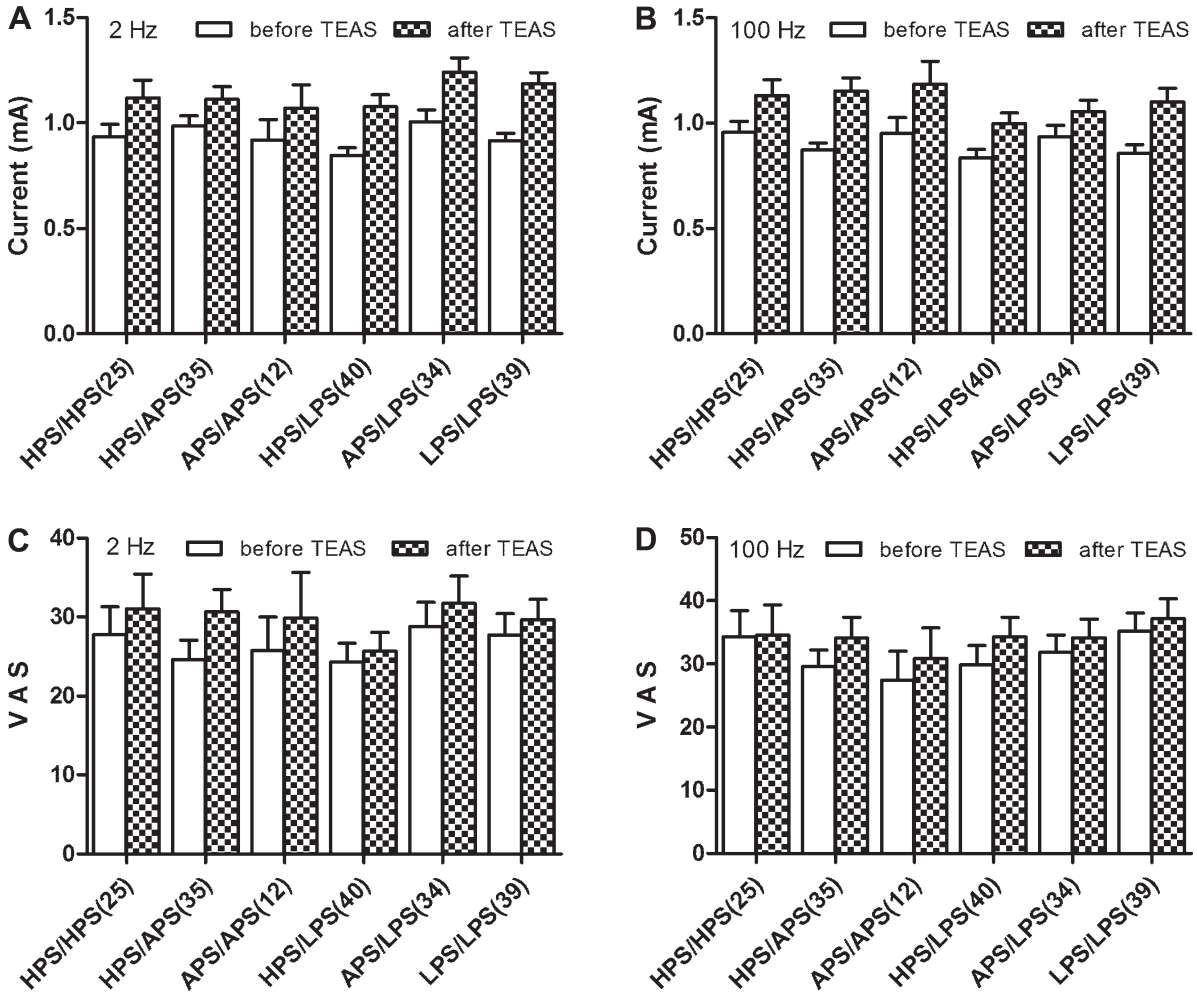

Fig. 3. Pain responsiveness (*A*, 2 Hz; *B*, 100 Hz) and VAS ratings in response to the 2-fold of pain threshold stimulation (*C*, 2 Hz; *D*, 100 Hz) are categorized according to the 6 COMT haplotype combinations. Low pain sensitive (LPS) haplotype, GCGG; average pain sensitive (APS) haplotype, ATCA; high pain sensitive (HPS) haplotype, ACCG. Lower current values reflect greater pain sensitivity in the pain threshold, and lower VAS ratings reflect less sensitivity to the same intensity stimulus. Each value represents the mean pain threshold or VAS rating with the associated SE. Numbers in parentheses indicate participants' genotype numbers. See also Figs. 4–9.

Genotyping the COMT locus. Genomic DNA was purified using the TIANamp blood DNA Kit (TIANGEN Biotech, Beijing, China). Five COMT SNPs (rs6269, rs4633, rs4818, rs165599, and rs4680) were tested using SNaPshot as previously described (31, 37, 41). A segment of DNA surrounding the SNPs (100 bp) was amplified by PCR using HotStarTaq (Qiagen). After purification using shrimp alkaline phosphatase and exonuclease I (Epicentre), the PCR products were subjected to a primer extension assay using the SNaPshot Multiplex kit (ABI), which adds a single fluorophore-labeled dideoxyribonucleotide triphosphate that is complementary to the nucleotide at the polymorphic site. The resulting primer extension products were analyzed on an ABI 3130xl capillary electrophoresis DNA instrument, using Gene Mapper 4.0 software (Applied Biosystems, Foster City, CA). The SNP locus rs2075507 was genotyped using PCR and restriction fragment length polymorphism analyses. The following primers were used for PCR: rs2075507F CTCCTCTGGCGGAAAGGAAT, and rs2075507R ATTCGGCATCAAAAGGAGGA. The fragments were separated on a 12% acrylamide gel and stained with ethidium bromide. Haplotype reconstructions were performed using the PHASE software program (33, 34), and our analysis was limited to subjects who carried one of the six major diplotypes (HPS/HPS, LPS/LPS, APS/APS, LPS/APS, LPS/HPS, or APS/HPS).

Statistical analysis. The data were processed using the commercially available software program GraphPad Prism 5.0. Pain thresholds and VAS ratings for the fixed twofold pain threshold stimulation before and after TEAS compared within the entire population are presented as means ± SE and analyzed by a paired *t*-test (Fig. 2). The associations of pain perception with each of the six SNPs, as well as the six combinations of the three most prevalent haplotypes were evaluated using a one-way ANOVA followed by Bonferroni post hoc tests (see Figs. 3–9). The TEAS analgesic effects were analyzed and associated with each of the six SNPs, and the six diplotypes using two-way ANOVA. The significance threshold was set to $P < 0.05$.

RESULTS

Pain sensitivity varies significantly in the human population. At the 2 Hz TEAS session, the basal pain threshold was 0.93 ± 0.02 mA (mean ± SE) and ranged from 0.33 to 1.50 mA. After 30 min of 2 Hz TEAS, the pain threshold increased significantly to 1.15 ± 0.03 mA and ranged from 0.50 to 2.00 mA (Fig. 2, *A* and *C*). At the 100 Hz TEAS session, the pain threshold was 0.89 ± 0.02 mA and ranged from 0.43 to 1.50 mA. After 30 min of 100 Hz TEAS, the pain threshold increased significantly to 1.10 ± 0.03 mA and ranged from 0.40 to 2.00 mA (Fig. 2, *B* and *C*). There were no significant differences found in the basal pain threshold between the two sessions (Fig. 2*C*). The VAS rating for the fixed twofold pain threshold stimulation increased significantly from 26.1 ± 1.1 on the first test to 29.6 ± 1.3, 31.4 ± 1.2, and

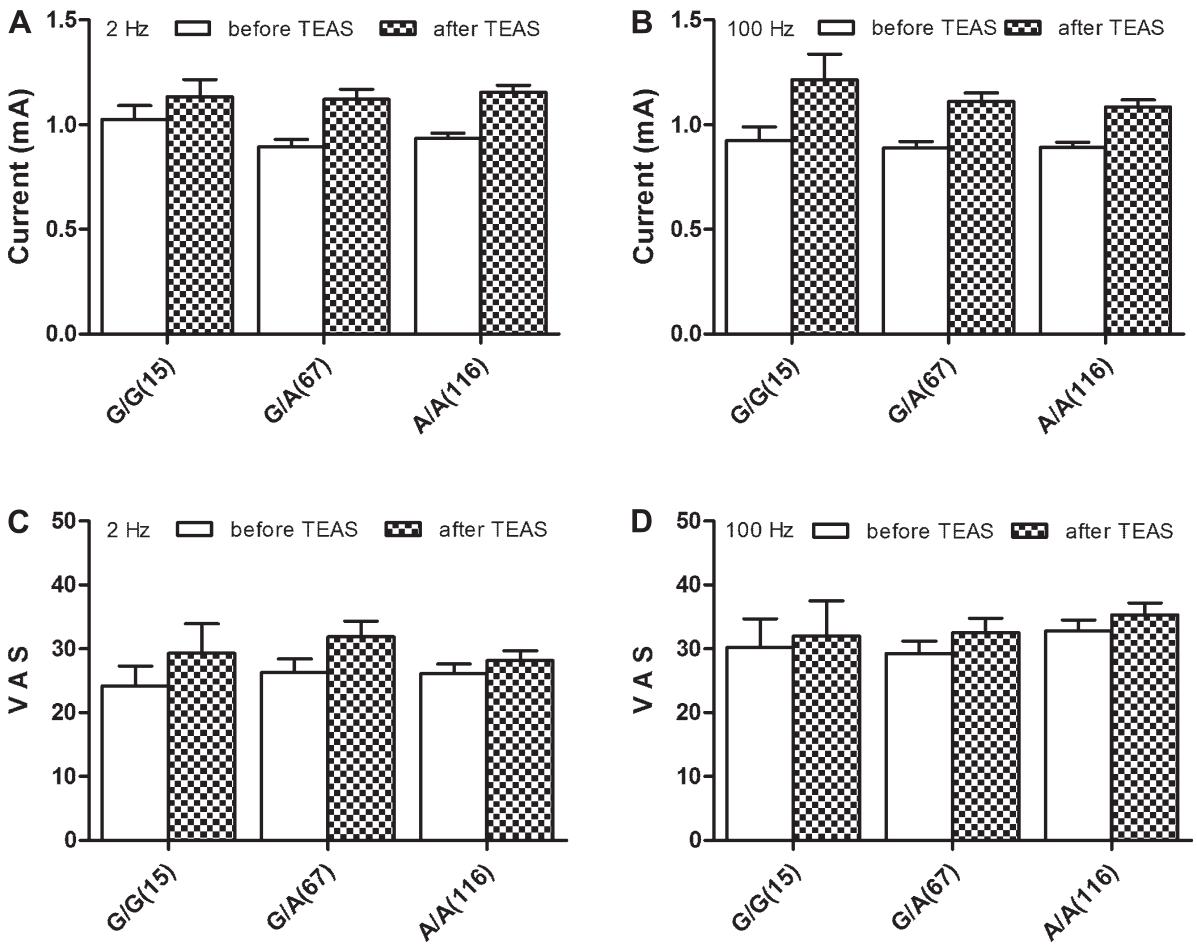

Fig. 4. The pain responsiveness (*A*, 2 Hz; *B*, 100 Hz) and VAS ratings in response to the fixed 2-fold of pain threshold stimulation (*C*, 2 Hz; *D*, 100 Hz) are categorized according to the 3 different genotypes of the rs2075507 SNP. Lower current values reflect greater pain sensitivity in the pain threshold, and lower VAS ratings reflect less sensitivity to the same intensity stimulus. Each value represents the mean pain threshold or VAS rating with the associated SE. No significant differences were observed in pain thresholds or VAS ratings among the genotypes.

34.1 ± 1.4 on the subsequent tests independent of the order of TEAS (Fig. 2*D*).

Genotyping of the COMT SNP polymorphisms. According to our genotyping results, the frequencies of the six SNPs minor alleles are consistent with the data stored in HapMap database (http://hapmap.ncbi.nlm.nih.gov/) and are listed in Table 1. Based on linkage disequilibrium analyses, SNPs have been found to cosegregate in haploblocks (1). According to Diatchenko's observation, four centrally located SNPs in the COMT gene, including rs6269, rs4633, rs4818, and rs4680, generated haplotypes. These polymorphisms gave rise to seven haplotypes with a frequency of >0.5%, all of which account for 96.75% of our results. Three haplotypes were designated as HPS, APS, or LPS (8). The six diplotypes of these haplotypes represented 92.5% of all haplotypes combinations observed in this study. The ACCG_ACCG combination (HPS/HPS) accounts for 13.5% of all six diplotypes. Similarly, the ATCA_ACCG combination (APS/HPS), the ATCA_ATCA combination (APS/APS), the ACCG_GCGG combination (APS/LPS), the ATCA_GCGG combination (APS/LPS), and the GCGG_GCGG combination (LPS/LPS) account for 18.9, 6.5, 21.6, 18.4, and 21.1% of all six diplotypes, respectively. Different to the results of Diatchenko's that only four subjects were homozygous for the HPS haplotype and excluded from analysis (8), we have 25 subjects homozygous for the HPS haplotype.

Effects of the COMT genetic polymorphisms on pain sensitivity and TEAS-induced analgesia. Our results indicate that the six haplotype combinations examined in this study (HPS/HPS, HPS/APS, APS/APS, HPS/LPS, APS/LPS, and LPS/LPS) have not significantly correlated with pain perception (Fig. 3). Five of the six polymorphic loci in the COMT gene (rs2075507, rs6269, rs4818, rs4680, and rs165599) examined here also lack a correlation with pain perception (Figs. 4, 5, 7–9). However, we did observe significant differences in pain threshold in subjects harboring the C/C and C/T genotypes at the rs4633 loci for the 2 Hz session, but not for the 100 Hz session (Fig. 6). The diplotypes of the haplotypes were not associated with the VAS ratings of the fixed twofold pain threshold stimulation (Fig. 3, *C* and *D*). None of the six polymorphic loci in COMT (rs2075507, rs6269, rs4633, rs4818, rs4680, rs165599) or the six haplotype combinations were significantly correlated with pain threshold changes induced by TEAS (Figs. 3-9).

DISCUSSION

This study investigated the effects of six COMT nucleotide polymorphisms and six haplotype combinations on pain per-

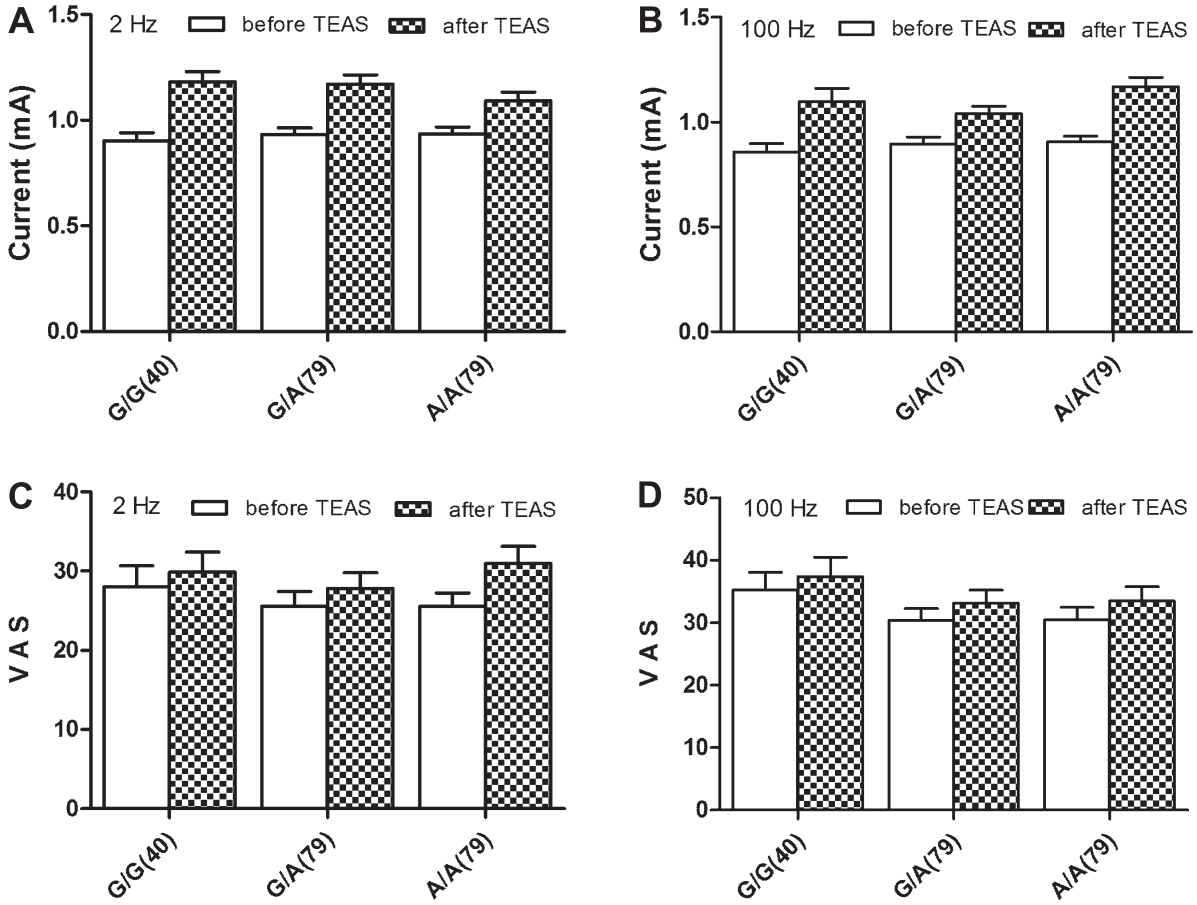

Fig. 5. The pain responsiveness (*A*, 2 Hz; *B*, 100 Hz) and VAS ratings in response to the fixed 2-fold of pain threshold stimulation (*C*, 2 Hz; *D*, 100 Hz) are categorized according to the 3 different genotypes of the rs6269 SNP. Lower current values reflect greater pain sensitivity in pain threshold, and lower VAS ratings reflect less sensitivity to the same intensity stimulus. Each value represents the mean pain threshold or VAS rating with the associated SE. No significant differences were observed in the pain thresholds or VAS ratings among the genotypes.

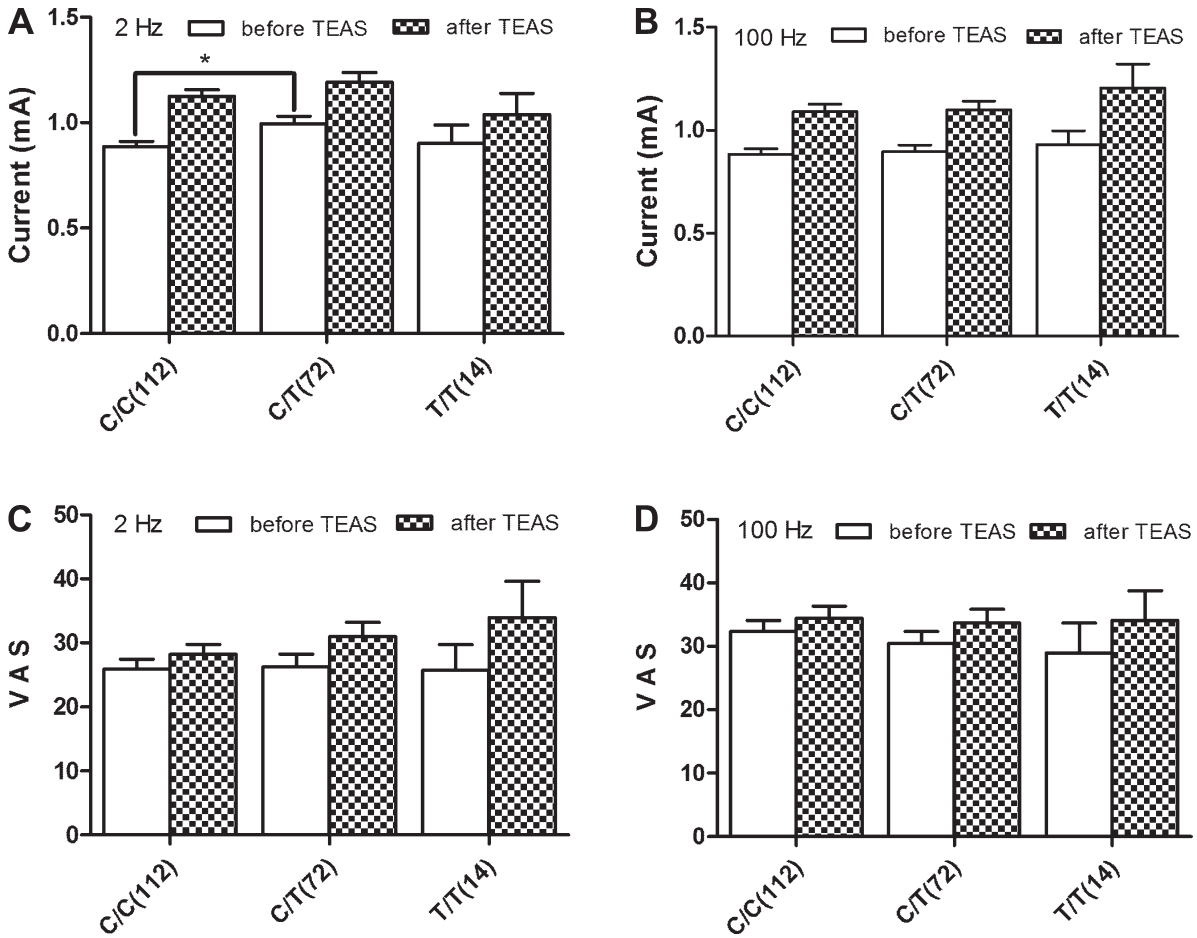

Fig. 6. The pain responsiveness (*A*, 2 Hz; *B*, 100 Hz) and VAS ratings in response to the fixed 2-fold of pain threshold stimulation (*C*, 2 Hz; *D*, 100 Hz) are categorized according to the 3 different genotypes of rs4633 SNP. Lower current values reflect greater pain sensitivity in the pain threshold, and lower VAS ratings reflect less sensitivity to the same intensity stimulus. Each value represents the mean pain threshold or VAS rating with the associated SE. There is significant difference (*A*) in pain threshold between genotypes of C/C and C/T (1-way ANOVA followed by a Bonferroni post hoc test). $*P < 0.05$.

ception and TEAS-induced analgesia. We found that the COMT gene polymorphisms and haplotypes studied here were not significantly associated with pain perception or TEAS-induced analgesia.

Effects of COMT gene polymorphisms on pain perception. To investigate the relative importance of genetic factor and measurement condition on human pain perception, we measured the basal pain threshold of each participant twice, once during a 2 Hz TEAS session and once during a 100 Hz TEAS session. No significant differences were observed between the average basal pain thresholds measured during these two sessions, which were 1 mo apart. Among all of the polymorphisms loci and six haplotype combinations, the only significantly different basal pain threshold was observed for the rs4633 locus in the first session. However, no dose effect relation was observed because of the fact that heterozygotes exhibited the maximum pain threshold. Thus, this result is more likely to be a random measurement error than a true effect of the genotype polymorphisms. The average VAS ratings of the fixed pain reported by subjects gradually and significantly increased from the first to the last test, despite the fact that the stimulus was held constant at the twofold pain threshold. This trend was observed regardless of the six gene loci and haplotypes polymorphisms. These results suggested that the COMT polymorphisms do not play an important role in pain perception ratings.

Although growing evidence suggest a significant role of COMT polymorphisms or COMT inhibition in determining pain sensitivity (7, 8, 26), the exact mechanism by which COMT activity influences pain perception is poorly understood. There are several potential mechanisms by which COMT activity could influence pain perception. For example, recent studies have demonstrated a central role for dopaminergic neurotransmission in modulating pain perception and basal pain thresholds (3), a finding that is consistent with observations made from several studies that investigated experimental pain (1). Nackley and colleagues reported that low COMT activity leads to increased pain sensitivity via a β_2- and β_3-adrenergic mechanism in rats (26). Zubieta et al. (43) have proposed that the rs4680 polymorphism influences pain sensitivity by indirectly regulating μ-opioid receptor function. Thus, the precise mechanisms by which reduced COMT activity leads to increased pain sensitivity require further elucidation and additional research.

It is likely that the observed differences in the results from published studies stem from the fact that pain perception is a complex process that is influenced by a variety of environmen-

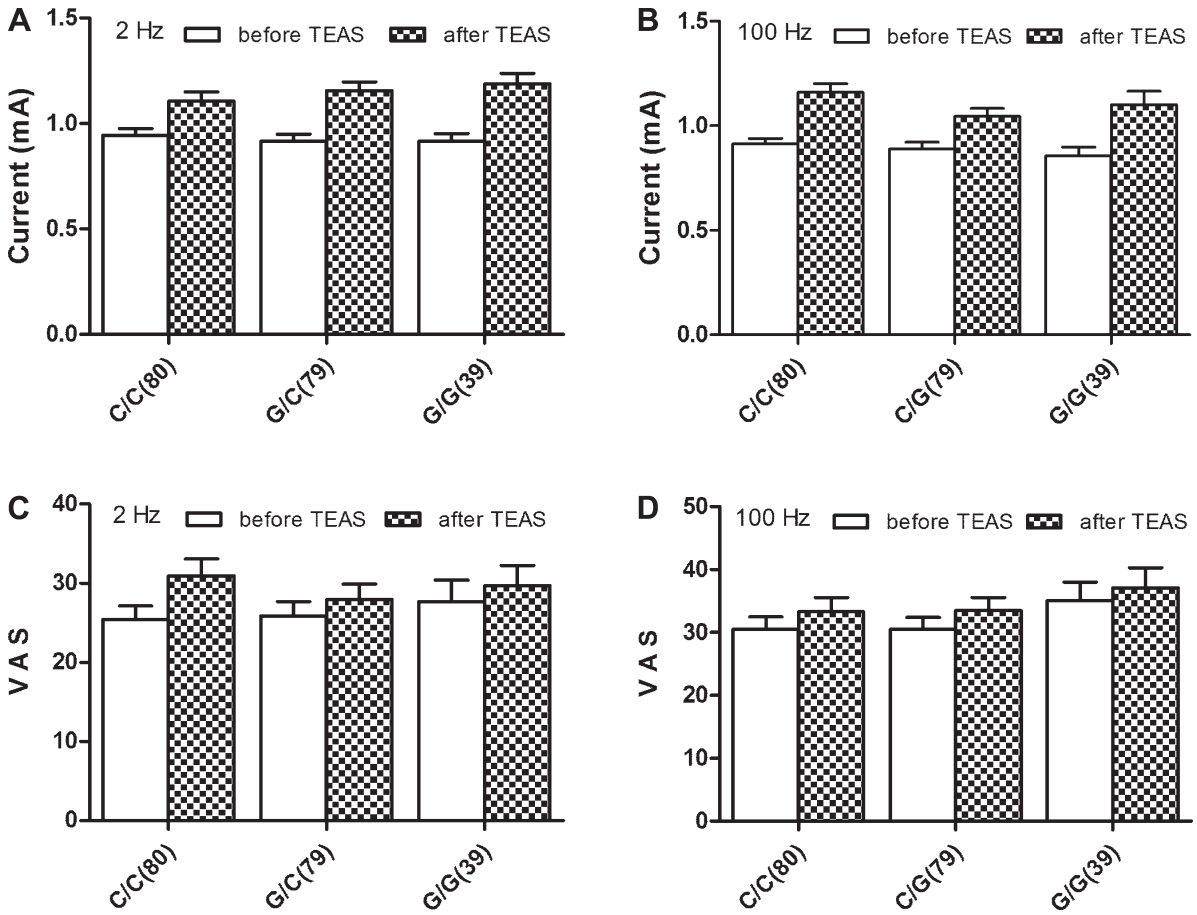

Fig. 7. The pain responsiveness (*A*, 2 Hz; *B*, 100 Hz) and VAS ratings in response to the fixed 2-fold of pain threshold stimulation (*C*, 2 Hz; *D*, 100 Hz) are categorized according to the 3 different genotypes of SNP rs4818. Lower current values reflect greater pain sensitivity in the pain threshold, and lower VAS ratings reflect less sensitivity to the same intensity stimulus. Each value represents the mean pain threshold or VAS rating with the associated SE. No significant differences in pain thresholds or VAS ratings were observed among the genotypes.

tal and genetic factors. The reported heritability for nociceptive and analgesic sensitivity is estimated to range from 28 to 76% in mice, but the relative importance of genetic versus environmental factors in human pain perception remains unclear (25). A highly significant association between COMT gene polymorphisms and pain perception has been reported by a previous study with a large population size (8). However, studies examining the associations between COMT gene polymorphisms and pain perception have also generated conflicting results. Kim and colleagues (20) found no association between the rs4680 SNP genetic polymorphism and the variations in heat and cold pain sensitivity in humans. Similarly, Nicholl and colleagues (27) found no evidence of an association between the COMT pain sensitivity haplotypes and chronic widespread pain in two population-based cohorts. Two large population-based studies have also suggested that the rs4680 locus does not play a role in neuropathic pain susceptibility in a Spanish population (2) or in chronic musculoskeletal complaints in Norway (11).

One possible explanation for the weak relationship between the SNPs or haplotype combinations and pain perception in the current study may be due to the ethnic population examined. The size and frequencies of haplotype blocks differ between different ethnic populations. Therefore, mixed populations may not be appropriate for the identification of SNPs or haplotypes that associated with pain phenotypes (19). However, the current study focused on a single ethnic population and still found no evidence of a relationship between the SNPs or haplotype combinations and pain perception. Sex may provide a second possible explanation. Fijal and colleagues (10) found that the baseline pain levels measured by VAS appeared to be associated with the COMT haplotypes only in female patients with major depressive disorder, but not in male patients. The rs165599 SNP did not affect baseline pain in either sex. Sex differences could potentially be explained by the lower baseline COMT protein levels in women, which could make them more susceptible to the impact of genetic polymorphisms on COMT translation and enzymatic activity compared with men. Indeed, there is evidence that the effects of these polymorphisms may be more biologically significant in women than in men (10). Thus, the lack of significant associations observed here could partially result from the inclusion of only male participants in our sample. A third potential explanation may be the method used to measure the pain threshold, the potassium iontophoresis method, which produces chemically induced pain. It is possible that results obtained with this method could be different compared with results obtained using other pain modalities.

As for the observed increase in VAS ratings that occurred with the sequence of the measurement, we speculate that this

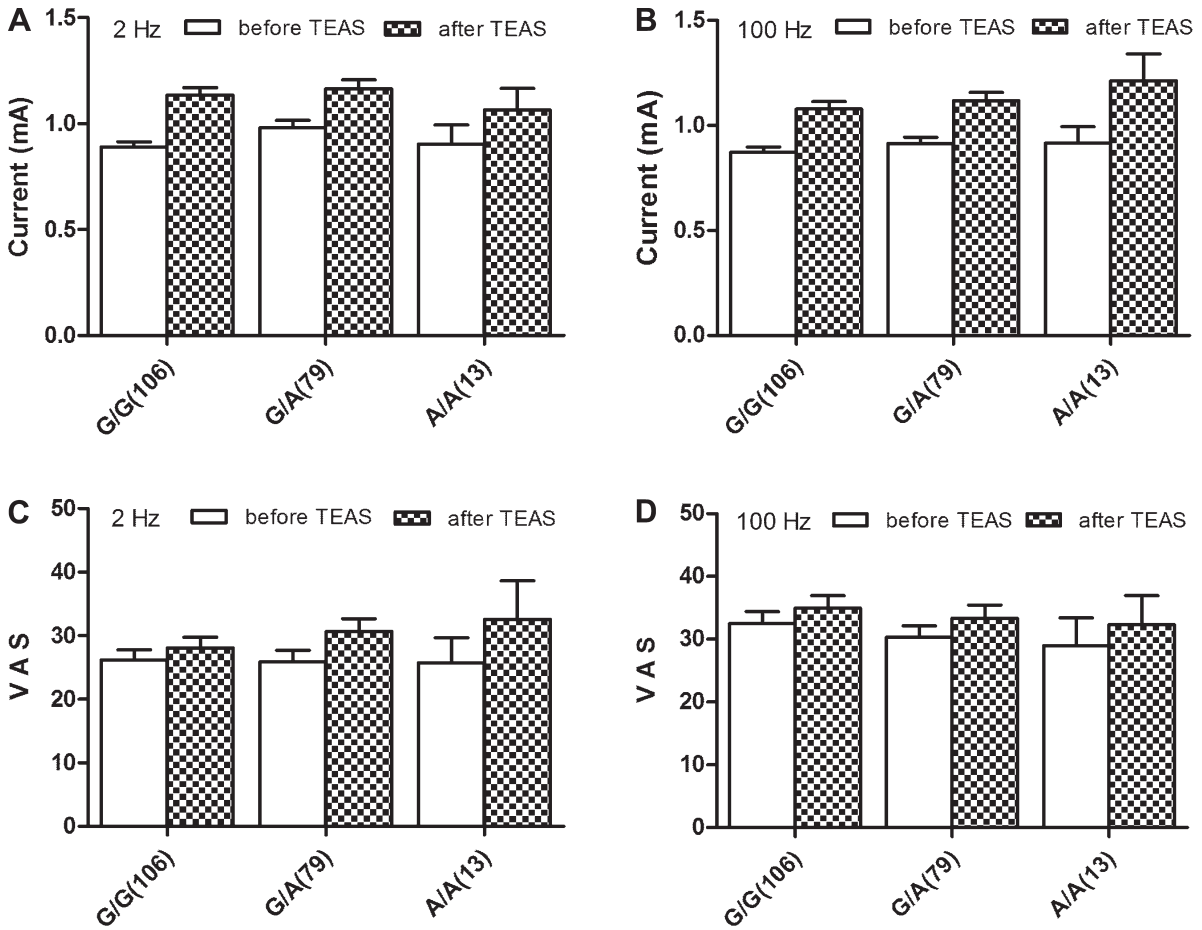

Fig. 8. The pain responsiveness (*A*, 2 Hz; *B*, 100 Hz) and VAS ratings in response to the fixed 2-fold of pain threshold stimulation (*C*, 2 Hz; *D*, 100 Hz) categorized according to the 3 different genotypes of SNP rs4680. Lower current values reflect greater pain sensitivity in the pain threshold, and lower VAS ratings reflect less sensitivity to the same intensity pain. Each value represents the mean pain threshold or VAS rating with the associated SE. No significant differences in pain thresholds or VAS ratings were observed among the genotypes.

phenomenon results from the summation of the pain (7) or pain experience (35, 36). Pain investigations are very complex because of the different levels of pain that can affect a patient's experience, especially with the use of analgesic drugs and different pain scales (36). Compared with measurements of pain threshold, which involve minimal pain, the fixed twofold threshold pain stimulation for 30 s is a moderate pain that can induce affective response. The affective dimension of the pain stimulation can be an important influencing factor resulting in increased VAS ratings (32). In contrast to Diatchenko's results, which showed that the rs4680 allele was associated with the temporal summation of pain (7), we found no relationship between the summation of pain perception and the COMT gene polymorphisms in six loci and their haplotype combinations.

Effects of COMT gene polymorphisms on TEAS-induced analgesia. Our previous studies have indicated that the release of endogenous opioid peptides in the central nervous system plays a key role in EA and peripheral electrical stimulation-induced analgesia (13). One purpose of our TEAS experiment was to test whether genotypes characterized by low COMT activity result in a weak TEAS analgesia phenotypes, which could potentially explain why some people do not respond to EA. This is based on the hypothesis of a tonic-phasic regulation of DA transmission in the nucleus accumbens and prefrontal cortex as previously described by Andersen and Skorpen (1). However, we found no associations between COMT genes or haplotype combinations polymorphisms and TEAS analgesia.

The other purpose of the TEAS experiment was to validate the effectiveness of the method used for pain measurement. Potassium iontophoresis is an effective, convenient, and reliable stimulus for experimental pain that allows for investigations into the neural modulation of pain in the relative absence of inflammatory processes and tissue damage, even at high stimulus intensities (18). Pain thresholds have repeatedly been shown to increase significantly after 2 or 100 Hz TEAS. Thus, we used TEAS analgesia as a positive control to demonstrate that the resolving power of this apparatus was sufficiently sensitive to distinguish potentially significant differences.

Limitations and perspective on the association of COMT activity and pain perception. Pain perception is a complex process that should be considered as both a sensation and an emotion. It shows considerable subjectivity, especially compared with other sensations (25). There are many confounding factors in studying the association of functional COMT variants and pain perception. First, it should be taken under

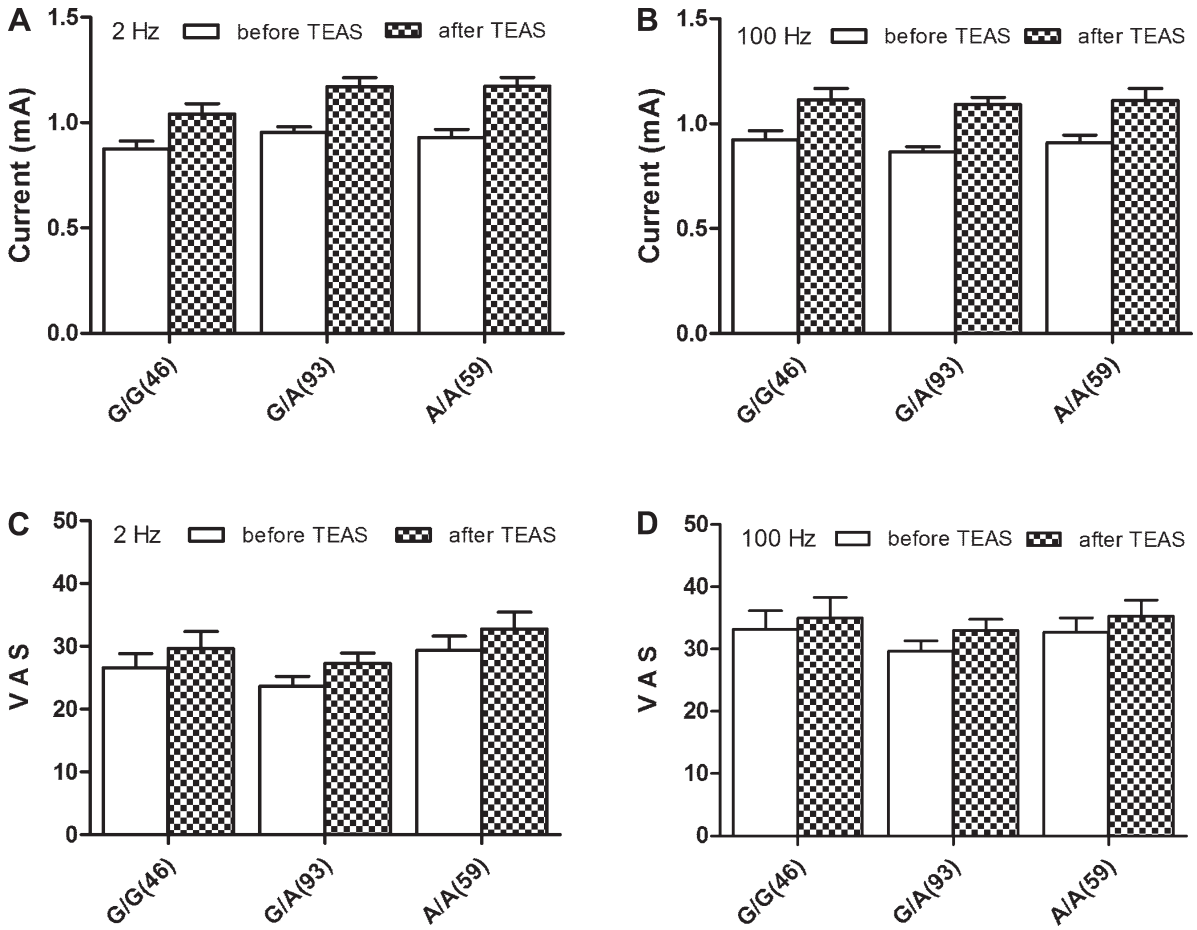

Fig. 9. The pain responsiveness (*A*, 2 Hz; *B*, 100 Hz) and VAS ratings in response to the fixed 2-fold of pain threshold stimulation (*C*, 2 Hz; *D*, 100 Hz) are categorized according to the 3 different genotypes of SNP rs165599. Lower current values reflect greater pain sensitivity in the pain threshold, and lower VAS ratings reflect less sensitivity to the same intensity stimulus. Each value represents the mean pain threshold or VAS rating with the associated SE. No significant differences in pain thresholds or VAS ratings were observed among the genotypes.

consideration that haplotype frequencies and block structures might be different between different ethnic groups, a fact that is particularly important when analyzing mixed ethnic samples. For example, the frequencies of the low activity allele at rs4680 in diverse populations vary significantly from 0.01 to 0.62 (29). Previous data have shown that ethnic differences in both pain perception and pain rating (4, 9). Because of this, populations in genetic association studies should ideally be ethnically homogeneous and preferably from a geographically defined region (28). In addition to ethnicity, sex, age, medication, and menstruation are factors known to be associated with chronic pain conditions or pain sensitivity and may act as potential confounders to any genetic association observed (1). Some inconsistencies are likely to result from the inability to adjust for the joint occurrence of confounding genetic variants in other genes that may occur when multiple loci act in concert to affect pain perception, which may vary between study populations (22). Smallness of the sample size is also an important confounding factor for the conflict results of different genetic association studies. In this study, we performed an experiment in a homogeneous ethnic population with participants of the same sex and within a narrow age range. Our primary limitations were the size of our sample and the low frequency of some of the SNPs employed, so we tested the pain threshold of the participants twice to compensate the limitations of this study. To minimize any differences between the VAS pain ratings for 2 and 100 Hz TEAS, either 2 or 100 Hz TEAS first should have been randomized to eliminate the effect of session order. However, we tested the 2 Hz session first based on the assumption that 2 Hz TEAS-induced analgesia is mediated by the μ-opioid receptor (13) and is more easily affected by COMT variants (43). The lack of randomization for the two sessions did not appear to influence the basal pain threshold or measurement conditions of the VAS pain ratings of each session because the stimuli were found to exhibit no differences other than the measurement sequence. However, it would have been more convincing if we had used a sham TEAS group to prove the effectiveness of TEAS analgesia. Our previous study showed that the verum TEAS group had an analgesic effect compared with the sham group (40).

We are unable to comment further on whether the previous reports of association are false-positives or due to real differences in phenotype or study design. Standard studies with uniform measurement methods and larger sample sizes and homogeneous ethnic populations are needed to verify or amend these preliminary results.

Conclusions

In summary, our results further confirm that TEAS exhibits a significant analgesic effect. However, we found no evidence indicating that COMT polymorphisms influence pain perception or TEAS-induced analgesia in humans. These results suggest that COMT activity may not to play a significant role in pain perception or TEAS-induced analgesia. Further endophenotyping studies of COMT gene polymorphisms are required to assess the potential correlations between COMT activity and human pain perception.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: X.X., J.H., and C.C. conception and design of research; X.X., Y.J., Y.N., M.F., F.S., and X.W. performed experiments; X.X., Y.J., and C.C. analyzed data; X.X. and C.C. interpreted results of experiments; X.X., X.W., and C.C. prepared figures; X.X. drafted manuscript; X.X., M.F., F.S., X.W., J.H., and C.C. edited and revised manuscript; X.X. and C.C. approved final version of manuscript.

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