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A variant in the *SCN10A* enhancer may affect human mechanical pain sensitivity

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Abstract

Expression of Nav1.8, encoded by *SCN10A*, can affect pain transmission and thus mediate the human pain phenotype. In the current study, we assessed whether the variant rs6801957, located in the *SCN10A* enhancer region, may have the potential to affect human pain. Through dual-luciferase reporter assays in 293T cells, we found that the *SCN10A* enhancer A (Enh-A) increased the activity of the *SCN10A* promoter ($P < 0.05$). Additionally, in a cohort of 309 healthy women, mutant rs6801957 A/A was found to have a significant association with decreased human experimental mechanical pain sensitivity ($P < 0.05$). We then found that mutant genotype A/A suppressed the increased effect of Enh-A compared with wild-type G/G ($P < 0.05$). The association between rs6801957 and human experimental mechanical pain sensitivity was further validated in a larger cohort of 1005 women ($P < 0.05$). In conclusion, these results demonstrated that the variant rs6801957 and Enh-A may affect *SCN10A* gene expression and play an important role in human mechanical pain sensitivity.

Keywords

SCN10A, Nav1.8, pain sensitivity, enhance

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Introduction

Nav1.8 sodium channel, encoded by *SCN10A*, is associated with human peripheral painful neuropathy[1] and is preferentially expressed in peripheral nociceptive neurons, particularly the dorsal root ganglion.[2–4] Based on the biophysical properties of Nav1.8 and its critical role in repetitive firing, Nav1.8 is thought to affect nociceptor excitability, thereby contributing to pain.[5–8] Indeed, previous studies have demonstrated that changes in Nav1.8 expression play an important role in diverse pain phenotypes, including inflammation and neuropathic pain.[9,10] Furthermore, in human studies, increases in Nav1.8 expression have been found in patients with myofascial temporomandibular disorders or lingual nerve neuromas.[11,12] Thus, regulating Nav1.8 expression can affect pain transmission and the human pain phenotype, and exploring the underlying regulating mechanism may provide effective target for pain relief.

In recent years, genome-wide association studies of electrocardiogram measures in diverse populations have consistently shown associations between the voltage-gated sodium channel gene *SCN10A* and cardiac conduction system function.[13–15] Several single-nucleotide polymorphisms (SNPs) in *SCN10A* have been shown to have significant associations with cardiac conduction; however, at present, only rs6801957, located in enhancer A (Enh-A) within *SCN10A*, has been shown to have a direct correlation with *SCN5A* expression and thus influence cardiac physiology.[16] Using high-resolution chromatin conformation capture (4C), researchers demonstrated that Enh-A may have the potential to interact with neighbouring promoters, including the promoter of *SCN5A* and *SCN10A*.[17]

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And in the previous study,[16] the association between Enh-A and expression of *SCN5A* and its further effect on cardiac conduction have been demonstrated. Therefore, based on the critical role of *SCN10A* in nociceptor excitability and the potential of Enh-A to affect the expression of *SCN10A*, we hypothesized that Enh-A may also play an important role in modulation of human pain sensitivity. Accordingly, in this study at the mechanistic and association levels, we tested the hypothesis that this Enh-A variant could affect human pain.

Methods

Study design

First, we explored the effects of Enh-A on the activity of the *SCN10A* gene promoter by dual-luciferase reporter assays. After the role of Enh-A was identified, we analysed the association between rs6801957 (G>A), located in Enh-A of *SCN10A*, and pain sensitivity in a healthy volunteer population. Then, we evaluated the effects of Enh-A with rs6801957-A or rs6801957-G on the activity of the *SCN10A* gene promoter by construction of site-directed mutagenesis in vitro. Finally, the effects of rs6801957 on human pain sensitivity were verified in a larger sample population.

Construction of expression vectors

The positions of Enh-A and the *SCN10A* promoter sequence are shown in Figure 1(a). The fragment containing *SCN10A* Enh-A and promoter was directly amplified from human genomic DNA (Figure 1(b)). We then established a double-enzyme digestion system: *Mlu*I enzyme plus *Kpn*I enzyme plus mutant *SCN10A* Enh-A, *Mlu*I enzyme plus *Kpn*I enzyme plus the GL3 promoter plus GL3 basic, *Xho*I enzyme plus *Kpn*I enzyme plus GL3 basic vector and *Xho*I enzyme plus *Kpn*I enzyme plus *SCN10A* promotor plus GL3 basic vector. Next, the expression vector was constructed using the above double-enzyme digestion system (Figure 1(c)): (1) GL3 basic, (2) *SCN10A* promoter plus GL3 basic and (3) Enh-A plus *SCN10A* promoter plus GL3 basic. The products were transformed into

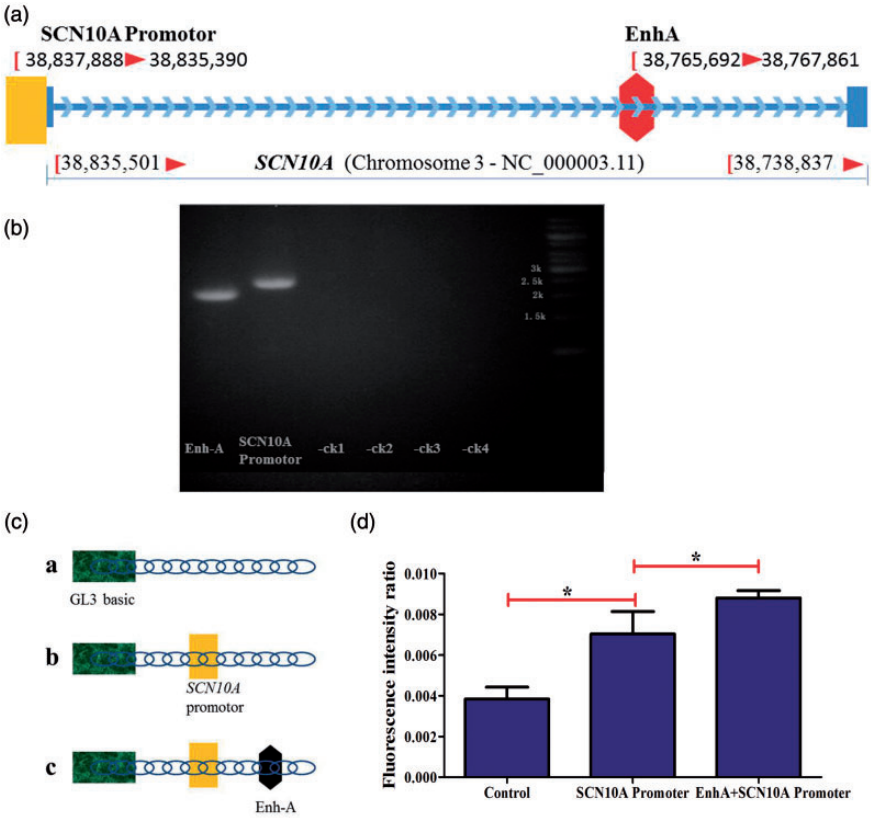

Figure 1. Effects of human Enh-A on the activity of the *SCN10A* promotor. (a) Spatial positions of the *SCN10A* promoter and Enh-A. (b) The fragments containing *SCN10A* Enh-A (about 2000 bp, the first lane) and *SCN10A* promoter (about 2500 bp, the second lane) were directly amplified from human genomic DNA. Lanes one to seven represent EnhA, *SCN10A* promotor, blank control 1, blank control 2, blank control 3, blank control 4 and marker, respectively. (c) Different expression vectors were constructed. (d) The fluorescence intensity ratios of different vectors ($n = 6$, $^*P < 0.05$).

competent cells, and the plasmids were extracted and validated by sequencing.

The concentrations of these three vector plasmids were aligned with the assay plasmid. On the day before transfection, the cells were inoculated in 24-well plates (1×10^5 cells/well) and 1 mL Dulbecco's modified Eagle's medium (DMEM; high-glucose) containing 5% foetal bovine serum. When cells reached 70% to 90% confluence, the three vector plasmids (containing GL3 basic, *SCN10A* promoter plus GL3 basic or Enh-A plus *SCN10A* promoter plus GL3 basic) were transfected into 293T cells via Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA) in Opti-MEM according to the manufacturer's instructions. One microliter of the RL-TK plasmid was added to serum-free Opti-MEM, and cells were incubated at 37°C for 5 h, after which the medium was replaced with fresh medium. Cells were then cultured for an additional 48 h.

Dual-luciferase reporter assay

According to the protocol of the Dual-Luciferase Reporter Assay System kit, we prepared passive lysis buffer (1× PLB), LAR II and Stop & Glo Reagent and lysed the cells. We then examined the fluorescence values of these three plasmids using a GloMax 20/20 Luminometer (Promega, Madison, WI). Finally, the fluorescence ratio of each plasmid was calculated from the reading of GL3 and RL-TK (fluorescence detection value of GL3 basic/fluorescence detection value of internal reference RL-TK).

Site-directed mutagenesis

Through comparative analysis of *SCN10A* gene sequences from different species, we found that the site of rs6801975 was highly conserved (Figure 2(a)); thus, this site may be functional and affect the activity of Enh-A. Point mutations in the plasmid were made based on the above plasmid *SCN10A* Enh-A plus *SCN10A* promoter plus GL3 basic; the wild-type sequence was changed to the mutant type, which was transformed into competent cells, and the wild-type plasmid was extracted and verified by sequencing. In the same way, the effects of the mutant plasmid on the activity of the *SCN10A* gene promoter were evaluated by dual-luciferase reporter assays.

Participants

We enrolled 309 female undergraduates and 1025 patients in this study. All participants were of Chinese Han ethnic origin and were recruited in our previous studies, which were designed to explore the associations

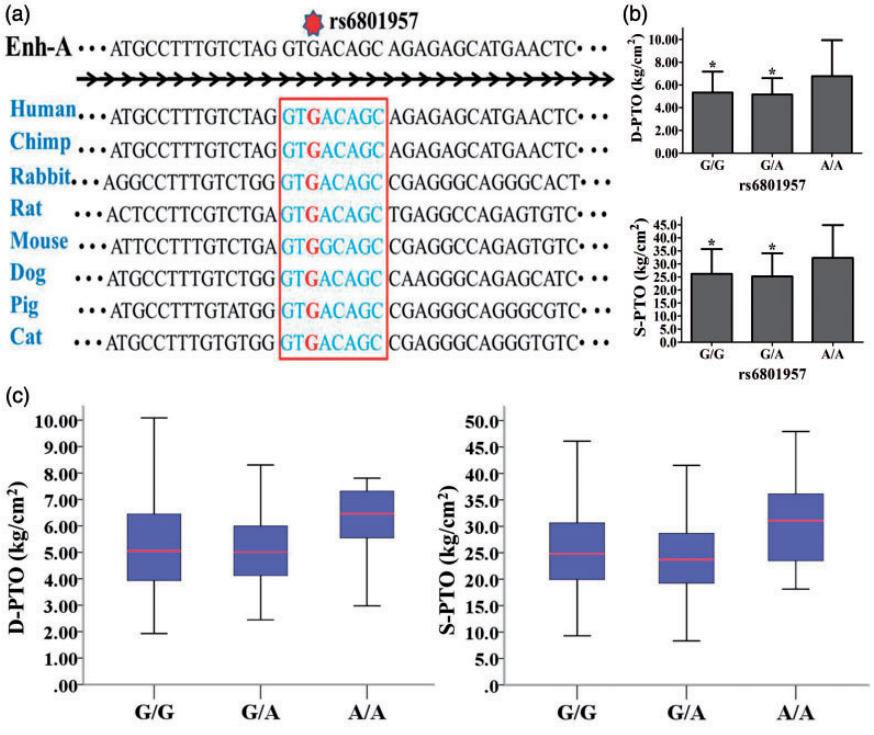

Figure 2. Conserved properties of Enh-A across different species (a) and the mean value (b) and distribution (c) of dull pressure pain tolerance (D-PTO) and sharp pressure pain tolerance (S-PTO) for healthy individuals with different rs6801957 alleles. $^*P < 0.05$; red lines represent medians; square boxes represent interquartile ranges; T bars represent the 5% to 95% line.

between some gene variants and human experimental pain sensitivity.[5,18] The study protocols were approved by the Institutional Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, and all methods were performed in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the study.

The inclusion and exclusion criteria were uniform for the two populations. The inclusion criteria were Chinese Han ethnicity, right-hand dominant. The exclusion criteria were as follows: incapable of communicating, smoking, alcohol use, drug abuse, pregnancy or menstrual period time, and use of any analgesic medication over the previous four weeks.

Human experimental pain test

For the 309 healthy women enrolled in this study, experimental mechanical and heat pain measurements were performed to explore the possible effects of the SNP rs6801957 on human pain. For participants in the primary study, we measured D-PPT, D-PTO, S-PPT, S-PTO and quantitative pricking pain threshold (QPT) to assess mechanical pain sensitivity. Withdrawal latency time (WLT) was measured to evaluate heat pain sensitivity.[19] For the larger population of 1025 women, who all were scheduled for elective gynaecological surgery, experimental pain tests were performed in the gynaecology ward prior to surgery. For this population, only mechanical pain sensitivity, including D-PPT, D-PTO, S-PPT and S-PTO, were analysed, and the test procedure was same as that in the primary study.[5]

A hand-held electronic mechanical algometer was used to test mechanical pain sensitivity, including D-PPT and D-PTO, with a 1-cm^2 probe, while sharp pressure pain sensitivity (S-PPT and S-PTO) were measured using a 0.1-cm^2 probe. Finally, the QPT was measured using a 0.01-cm^2 probe. The investigator applied the algometer to each of three locations on the right forearm in the following sequential manner: location 1 (the lateral brachioradialis of the elbow joint) for dull pressure pain sensitivity measurement; location 2 (the midpoint between locations 1 and 3) for sharp pressure pain sensitivity measurement and location 3 (the midpoint of the medial and lateral borders of the wrist) for QPT. A standardized procedure was used to test mechanical pain sensitivity for all participants. Participants were asked to say 'pain' when they started to feel pain (D-PPT, S-PPT or QPT) during the stimulation; the stimulation was restarted after a brief pause following this statement, and the participants were asked to state 'okay' when the pain became intolerable (D-PTO or S-PTO). Each pain test was repeated 5 min later, and the average of the two measurements was calculated. The order of the pain tests was identical for all participants.

WLT was measured using an Ugo Basile Biological Apparatus (model 37370; Ugo Basile, Varese, Italy) with infrared radiance intensity set at 60. The participants positioned their middle finger above the infrared generator. The investigator then activated both the infrared source and a reaction time counter via a start key. Participants were told to withdraw their finger when they started to feel pain; at this point, the infrared beam was automatically switched off, and the timer stopped. The left and right middle fingers of each participant were measured, and the average WLT of the two measurements was calculated.

Genotyping of rs6801957

We collected heparin-treated blood for all participants from the antecubital vein. Genomic DNA was extracted from blood samples using the guanidinium isothiocyanate method. Genotyping of rs6801957 was performed by Shanghai BioWing Applied Biotechnology Company (http://www.biowing.com.cn/) using ligase detection reactions (LDRs). The target DNA sequences were amplified using a multiplex polymerase chain reaction method. After completion of the amplification, the ligation reaction for each participant was carried out, and LDR was performed using 40 cycles of 94°C for 30 s and 63°C for 4 min. The fluorescent products of LDR were differentiated using an ABI sequencer 377.

Statistical analysis

All variables were summarized using standard descriptive statistics, such as the mean, standard deviation and frequency. Samples were tested for each SNP to determine whether the null hypothesis of the HWE could be rejected by applying the chi-square method. In the mechanism study in 293T cells, independent-sample t tests and one-way ANOVA with post hoc least-significant difference tests of quantitative traits were conducted. In the gene association studies in human populations, the primary healthy sample was taken as exploratory analysis, and thus statistical correction for six different experimental pain phenotypes was not performed. However, in the larger replication sample, Bonferroni correction test for five different pain phenotypes was performed. All analyses used the maximum number of cases available for each experimental pain phenotype. SPSS Statistics Version 17.0 (SPSS Statistics, Inc., Chicago, IL) was used, and a two-tailed probability value of less than 0.05 was used as the criterion for statistical significance.

Results

Effects of Enh-A on the activity of the SCN10A promoter

The spatial positions of the *SCN10A* promoter and Enh-A are shown in Figure 1(a), and the sequences of these regions were directly amplified from human genomic DNA. Different expression vectors were then constructed, as shown in Figure 1(c), and were transfected into 293T cells. Dual luciferase assays were performed. The results (Figure 1(d)) showed that the fluorescence intensity ratio of the *SCN10A* promoter plus the GL3 basic plasmid (0.0070 ± 0.0011) was significantly higher ($P = 0.001$) than that of the GL3 basic plasmid alone (0.0038 ± 0.0006), suggesting that the *SCN10A* promoter could significantly increase the expression of the common gene GL3 basic. The fluorescence ratio of Enh-A plus the *SCN10A* promotor and the GL3 basic plasmid (0.0085 ± 0.0006) was significantly higher ($P = 0.041$) than that of the *SCN10A* promotor plus the GL3 basic plasmid, indicating that Enh-A could increase the activity of the *SCN10A* promoter and thereby promote the expression of the GL3 basic gene.

Association between rs6801957 and human experimental pain sensitivity in healthy individuals

As shown in Figure 2(a), rs6801957 was located in Enh-A and was conserved across different species. Based on the role of *SCN10A* in humans, we speculated that this SNP may affect human pain. Thus, we assessed experimental pain sensitivity, including mechanical and heat stimulus, in 309 healthy women. To control for the effect of gender on experimental pain sensitivity, only female subjects were included in the study. The success genotype screening rate for rs6801957 in this population was 0.987; thus, 305 individuals were included in the final analysis. According to the genotypes of rs6801957 alleles, this cohort was divided into three groups: 187 (61.3%) individuals were homozygous for the major allele G/G, 107 (35.1%) were heterozygous for alleles G/A and 11 (3.6%) were homozygous for the minor allele A/A. The Hardy–Weinberg equilibrium (HWE) value of rs6801957 for the primary cohort was $P = 0.365$.

As shown in Table 1 and Figure 2, significant differences were found in mechanical pain sensitivity, including dull pressure pain tolerance (D-PTO; analysis of variance (ANOVA) $P = 0.017$) and close to significant in sharp pressure pain tolerance (S-PTO; ANOVA $P = 0.059$), among different genotype groups. Individuals who carried the minor homozygote allele showed higher D-PTO (A/A, 6.77 ± 3.16 vs. G/A, 5.33 ± 1.84 ($P = 0.010$) and vs. G/G 5.16 ± 1.44 ($P = 0.004$), respectively; Figure 2(b)) and S-PTO (A/A, 32.3 ± 12.6 vs. G/A, 26.2 ± 9.5 ($P = 0.040$) and vs. G/G, 25.2 ± 8.9 ($P = 0.018$), respectively; Figure 2(c)) than those who carried major homozygous or heterozygous alleles. However, there were no significant differences (all $P > 0.05$) among the other three types of mechanical pain measures and heat pain measures. Thus, rs6801957 may affect human pain and *SCN10A* expression.

Effects of rs6801957 genotyping on SCN10A promoter activity

Next, through site-specific in vitro mutations and dual luciferase assays, we investigated the effects of rs6801957 on the activity of the *SCN10A* promoter. As shown in Figure 3(a) and (b), the fluorescence ratio of mutant Enh-A(A) plus the *SCN10A* promoter and GL3 basic plasmid was significantly lower than that of wild-type Enh-A(G) plus the *SCN10A* promoter and GL3 basic plasmid ($P = 0.019$), although there were no differences when compared with that of the *SCN10A* promoter plus GL3 basic plasmid ($P = 0.624$). This suggested that the

Table 1. Experimental pain data for healthy individuals with different genotypes of rs6801957.

	G/G (n = 187)	G/A (n = 107)	A/A (n = 11)	ANOVA *P*
Age (years)	22.6 ± 2.2	22.4 ± 2.2	22.4 ± 1.6	0.694
Height (cm)	160.8 ± 4.7	160.5 ± 4.7	159.6 ± 2.9	0.604
Weight (kg)	51.3 ± 5.9	50.7 ± 5.9	51.0 ± 5.2	0.722
BMI (kg/m^2)	19.8 ± 2.0	19.7 ± 1.8	20.1 ± 2.1	0.688
D-PPT (kg/cm^2)	2.86 ± 1.02	2.91 ± 0.98	2.90 ± 1.14	0.917
D-PTO (kg/cm^2)	5.33 ± 1.84*	5.16 ± 1.44*	6.77 ± 3.16	0.017
S-PPT (kg/cm^2)	13.3 ± 3.9	12.7 ± 3.1	13.0 ± 5.2	0.479
S-PTO (kg/cm^2)	26.2 ± 9.5*	25.2 ± 8.9*	32.3 ± 12.6	0.059
QPT (kg/cm^2)	43 ± 12	45 ± 15	46 ± 21	0.608
WLT (s)	6.0 ± 3.1	6.4 ± 3.6	5.7 ± 1.7	0.479

BMI: body max index; D-PPT: dull pressure pain threshold; D-PTO: dull pressure pain tolerance; S-PPT: sharp pressure pain threshold; S-PTO: sharp pressure pain tolerance; QPT: quantizing pricking pain; WLT: withdrawal latency time.
*$P < 0.05$ when G/G and G/A compared with A/A.

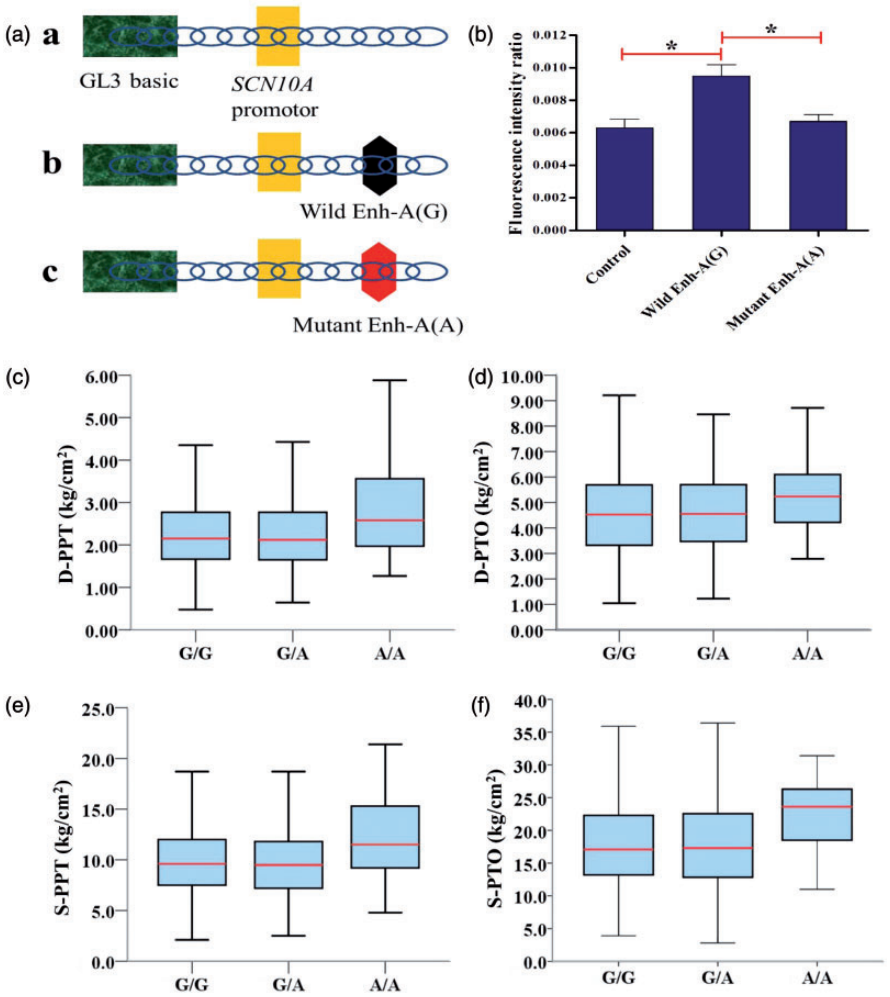

Figure 3. Effects of the rs6801957 genotype on the activity of the *SCN10A* promotor ((a) and (b), n = 6) and the distribution of experimental pain data for individuals with different rs6801957 alleles in the replication study ((c), dull pressure pain threshold; (d), dull pressure pain tolerance; (e), sharp pressure pain threshold; (f), sharp pressure pain tolerance). Red lines represent medians; square boxes represent interquartile ranges; T bars represent the 5% to 95% line. D-PPT: dull pressure pain threshold; D-PTO: dull pressure pain tolerance; S-PPT: sharp pressure pain threshold; S-PTO: sharp pressure pain tolerance.

interaction of wild-type Enh-A-G(G) with the *SCN10A* promoter was counteracted by mutant type Enh-A(A). Therefore, compared with the wild-type rs6801957, the mutant type may inhibit the activity of the *SCN10A* promoter, indicating that the mutant type rs6801957 may suppress *SCN10A* expression compared with the wild-type sequence.

Association between rs6801957 and human experimental pain sensitivity in women

We then assessed a larger sample population of 1005 women to validate the effects of rs6801957 on human experimental pain. In this cohort, the success genotype screening rate for rs6801957 was 0.954; thus, 959 women were included in the final analysis. According to the genotypes of rs6801957 alleles, the replication cohort was divided into three groups: 573 (59.7%) women were homozygous for the major allele G/G, 345 (35.9%) were heterozygous for alleles G/A and 41 (4.4%) were homozygous for the minor allele A/A. The HWE value of rs6801957 for the primary cohort was $P = 0.223$. For all individuals in the primary and replication studies, the minor allele frequency (MAF) was 0.220, and the frequency of minor homozygote was 0.041.

As shown in Table 2, there were significant differences in all types of dull and sharp pressure pain sensitivities among the different genotype groups (ANOVA, $P = 0.002$, 0.017, 4.1×10^{-5} and 0.001 for D-PPT, D-PTO, S-PPT and S-PTO, respectively). Individuals who carried the minor homozygote allele showed higher D-PPT (A/A, 2.86 ± 1.22 vs. G/A, 2.33 ± 0.97 ($P = 0.001$) and vs. G/G, 2.30 ± 0.96 ($P = 0.001$),

Table 2. Experimental pain data for women with different genotypes of rs6801957.

	G/G (n = 573)	G/A (n = 345)	A/A (n = 41)	ANOVA *P*	Bonferroni *P*
Age (years)	38.0 ± 11.1	38.9 ± 10.7	40.1 ± 11.7	0.306	NA
Height (cm)	160.3 ± 4.8	160.3 ± 4.9	160.3 ± 5.5	0.281	NA
Weight (kg)	57.4 ± 9.2	56.8 ± 8.9	59.1 ± 9.7	0.998	NA
BMI (kg/m^2)	22.3 ± 3.4	22.1 ± 3.4	22.9 ± 3.3	0.281	NA
D-PPT (kg/cm^2)	2.33 ± 0.97*	2.30 ± 0.96*	2.86 ± 1.22	0.002	0.010
D-PTO (kg/cm^2)	4.62 ± 1.68*	4.63 ± 1.65*	5.39 ± 1.59	0.017	0.085
S-PPT (kg/cm^2)	10.2 ± 4.1*	10.0 ± 4.3*	13.3 ± 6.3	4.1×10^{-5}	2.05×10^{-4}
S-PTO (kg/cm^2)	18.7 ± 8.2*	18.8 ± 8.4*	23.6 ± 8.3	0.001	0.005
QPT (kg/cm^2)	26 ± 12	26 ± 13	29 ± 11	0.307	1.000

NA: not applicable; BMI: body max index; D-PPT: dull pressure pain threshold; D-PTO: dull pressure pain tolerance; S-PPT: sharp pressure pain threshold; S-PTO: sharp pressure pain tolerance; QPT: quantizing pricking pain; WLT: withdrawal latency time.
*$P < 0.05$ when G/G and G/A compared with A/A.

respectively), D-PTO (A/A, 5.39 ± 1.59 vs. G/A, 4.62 ± 1.68 ($P = 0.005$) and vs. G/G, 4.63 ± 1.65 ($P = 0.007$), respectively), S-PPT (A/A, 13.3 ± 6.3 vs. G/A, 10.2 ± 4.1 ($P = 1.2 \times 10^{-5}$) and vs. G/G, 10.0 ± 4.3 ($P = 1.0 \times 10^{-5}$), respectively) and S-PTO (A/A, 23.6 ± 8.3 vs. G/A, 18.7 ± 8.2 ($P = 2.1 \times 10^{-4}$) and vs. G/G, 18.8 ± 8.4 ($P = 4.3 \times 10^{-4}$), respectively) than those who carried the major homozygous or heterozygous alleles. The mean increased percentage of pressure pain sensitivity for minor homozygotes compared with others ranged from 16.4% to 33%. Additionally, as shown in Figure 3 (d) to (f), the distributions of the four types of pressure pain measurement values in individuals with minor homozygous alleles were significantly different from those of other individuals.

Discussion

In this study, we found that Enh-A could enhance the activity of the *SCN10A* promoter. Through experimental pain sensitivity measurement in healthy women, we found that the rs6801957 mutant located in Enh-A could reduce mechanical pain sensitivity (including D-PTO and S-PTO) in the participants; then, using duel-luciferase reporter assays, we found that rs6801957 mutant may inhibit the activity of the *SCN10A* promoter. In a larger female cohort, we further demonstrated that the rs6801957 mutant significantly reduced mechanical pain sensitivity (including D-PPT, D-PTO, S-PPT and S-PTO).

Enh-A was initially found to be associated with down-regulation of *SCN5A* expression and further to mediate the deceleration of cardiac conduction[16]; however, based on the critical role of the sodium channel Nav1.8 encoded by the *SCN10A* gene in pain signaling,[4] Enh-A may also be involved in regulation of pain sensitivity. Dual-luciferase experiments showed that Enh-A can enhance the activity of the *SCN10A* promoter, which partly verified the current study hypothesis that Enh-A may have the potential to interact with *SCN10A* promotor and thus down-regulate the expression of *SCN10A* as its role in *SCN5A* expression. Next, we carried out site-directed mutagenesis of rs6801957 on the dual-luciferase expression vector Enh-A. Notably, the expression of mutant GL3 basic was significantly lower than that of the wild-type sequence. Thus, we speculated that wild-type Enh-A(G) may interact with the *SCN10A* promoter and increase its expression, whereas the rs6801957 mutant Enh-A(A) may counteract this interaction. These results further indicated that different genotypes of rs6801957 may affect the role of Enh-A.

Given that rs6801957 is located in Enh-A and is highly conserved, we propose that rs6801957 may play an important role in the regulation of pain sensitivity. Therefore, we measured mechanical and thermal pain sensitivity and collected blood samples from participants for genotyping in order to explore the association between rs6801957 and pain sensitivity. Interestingly, we found that individuals with mutant rs6801957 had lower mechanical pain sensitivity. Although the statistical correction was not performed in this exploratory analysis of relative small sized cohort, it indicated that Enh-A may have the potential to influence human mechanical pain sensitivity. Accordingly, we recruited a larger sample to further verify the effects of rs6801957 on pain sensitivity, and the results showed that the mechanical pain sensitivity of individuals with mutant rs6801957 was significantly lower than that of the other individuals. The mean increased percentage of pressure pain sensitivity for the minor homozygote compared with that of the other groups ranged from 16.4% to 33%. In summary, rs6801957 affected Enh-A activity and participated in the regulation of pain sensitivity in the general population, further demonstrating that Enh-A had a significant effect on the expression of *SCN10A* and human mechanical pain sensitivity in the general population.

As reported in previous studies, a high degree of inter-individual differences was observed in pain sensitivity and reporting of pain in the clinical setting,[20,21] and genetic variation has been shown to explain a significant portion of this variability.[22,23] Therefore, the current findings can help us better understand the effects of genetic variation on human pain perception.[24] Furthermore, through the current study, we identified a known enhancer sequence and an SNP that affected cardiac induction and could regulate the expression of *SCN10A*. Importantly, Nav1.8, encoded by *SCN10A*, has been shown to play a critical role in human pain.[25,26] Thus, our findings suggest a novel target site for pain intervention. However, further studies are needed to fully elucidate this mechanism in the future.

This study had several limitations. First, because the effects of gender on experimental pain sensitivity have been demonstrated in previous study,[27] it was anticipated that gender would be a confounding factor to the association between SNP and human pain sensitivity. Moreover, to control this confounding factor, only female subjects were included in the study. Further study including both the sex cohorts should be performed. Second, the cohorts included in the study was of a single ethnicity (Chinese Han), and the frequency of the rs6801957 homozygous mutant was 0.041; in contrast, in the 1000G database, the frequencies of rs6801957 homozygous mutant type have been reported to be as high as 4.8% in East Asian population, 11.5% in South Asians, 2.1% in Africans and 17.0% and 17.9% in Americans and Europeans, respectively. Thus, further studies are needed to assess differences in the roles of rs6801957 in other populations. In addition, based on the important role of mechanical pain sensitivity in the development of chronic pain[18,28] and the importance of *SCN10A* in chronic pain,[4] rs6801957 may also be associated with chronic pain. However, in the current study, the clinical pain phenotype was absent. Thus, further studies are required to assess the effects of Enh-A and rs6801957 on the clinical pain phenotype. Finally, although we observed significant associations of pain with Enh-A and rs6801957, the specific mechanisms underlying their effects on pain signal transmission remain unclear. Additional studies are needed to explore these mechanisms and establish techniques to target these mechanisms.

In summary, rs6801957 in Enh-A was found to contribute to regulating pain sensitivity in the general population. Additionally, the effects of rs6801957 and Enh-A on the activity of the *SCN10A* gene promoter were validated by dual-luciferase reporter assays. These results indicated that the variant rs6801957 in Enh-A may affect *SCN10A* gene expression and play an important role in human pain sensitivity. Furthermore, our findings provide a novel potential target for the regulation of human pain sensitivity.

Authors' Contributions

GD and JS conceived research, analysis data and wrote the main manuscript; NL, MZ and QW collected the blood sample; YZ and HZ collected data; SG, YY and PH performed the pain tests and conducted the experiment of dual-luciferase reporter assay, XZ conceived and supervised project, analysis data and edited manuscript. All the authors reviewed the manuscript.

Declaration of Conflicting Interests

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