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The serotonin receptor 2A (HTR2A) rs6313 variant is associated with higher ongoing pain and signs of central sensitization in neuropathic pain patients

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Abstract

Background: The serotonin receptor 2A (HTR2A) has been described as an important facilitation mediator of spinal nociceptive processing leading to central sensitization (CS) in animal models of chronic pain. However, whether *HTR2A* single nucleotide variants (SNVs) modulate neuropathic pain states in patients has not been investigated so far. The aim of this study was to elucidate the potential association of *HTR2A* variants with sensory abnormalities or ongoing pain in neuropathic pain patients.

Methods: At total of 240 neuropathic pain patients and 253 healthy volunteers were included. Patients were phenotypically characterized using standardized quantitative sensory testing (QST). Patients and controls were genotyped for *HTR2A* g.-1438G > A (rs6311) and c.102C > T (rs6313). Genotype-related differences in QST parameters were assessed considering QST profile clusters, principal somatosensory components and sex.

Results: There was an equal distribution of rs6313 and linked rs6311 between patients and controls. However, the rs6313 variant was significantly associated with a principal component of pinprick hyperalgesia and dynamic mechanical allodynia, indicating enhanced CS in patients with sensory loss (-0.34 ± 0.15 vs. $+0.31 \pm 0.11$ vs., $p < .001$). In this cluster, the variant allele was also associated with single QST parameters of pinprick hyperalgesia (MPT, $+0.64 \pm 0.18$ vs. -0.34 ± 0.23 $p = .002$; MPS, $+0.66 \pm 0.17$ vs. -0.09 ± 0.23, $p = .009$) and ongoing pain was increased by 30%.

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Conclusions: The specific association of the rs6313 variant with pinprick hyperalgesia and increased levels of ongoing pain suggests that the HTR2A receptor might be an important modulator in the development of CS in neuropathic pain.

Significance: This article presents new insights into serotonin receptor 2A-mediating mechanisms of central sensitization in neuropathic pain patients. The rs6313 variant allele was associated with increased mechanical pinprick sensitivity and increased levels of ongoing pain supporting a contribution of central sensitization in the genesis of ongoing pain providing a possible route for mechanism-based therapies.

1 | INTRODUCTION

Neuropathic pain arises after lesion or disease of the somatosensory system (https://www.iasp-pain.org). It is characterized by multiple somatosensory phenomena including positive signs like ongoing or evoked pain and negative signs like hypoesthesia (Baron et al., 2012; Maier et al., 2010). These signs can be attributed to interaction of several pathophysiological mechanisms, that is, an imbalance of peripheral or central sensitization (CS) and inhibitory mechanisms. Peripheral sensitization results from increased excitability of primary afferents (clinical correlate: localized heat hyperalgesia). CS (clinical correlates: dynamic mechanical allodynia, pinprick hyperalgesia) is caused by increased excitability of wide-dynamic-range neurons in the dorsal horn of the spinal cord (Baron et al., 2010).

Spinal nociceptive processing is modulated by facilitatory and inhibitory descending pathways originating in the brainstem. While increased descending inhibition protects the transition from acute to chronic pain, increased descending facilitation might promote chronification (Ossipov et al., 2010). Among others, descending control is mediated via serotonergic (5-HT) pathways (Millan, 2002). The 5-HT2 receptor (HTR2) is thought to play a crucial role in spinal pain modulation. While HTR2C might be involved in antinociceptive pathways, HTR2A is assumed to play a pronociceptive role resulting in increased spinal nociceptive transmission and CS (Aira et al., 2010; Rahman et al., 2011; Van Steenwinckel et al., 2009). This is supported by HTR2A protein expression being positively correlated with mechanical hypersensitivity in an animal model of HIV-induced neuropathy (Van Steenwinckel et al., 2008). While mechanical hyperalgesia was absent in HTR2A knock-out mice, it was reduced in heterozygous mice. Moreover, pain-related behaviour signs of mechanical hyperalgesia or allodynia are attenuated by HTR2A antagonism in other animal models of neuropathic pain (Cervantes-Durán et al., 2016; Nitanda et al., 2005; Sasaki et al., 2006).

Modulation of the HTR2A functionality and activity is poorly understood, although the association of single nucleotide variants (SNVs) in *HTR2A*, that is, rs6311 and rs6313, was investigated within studies concerning depression and chronic pain. *HTR2A* variants were reported to be associated with a higher depression score in female chronic pain patients (Lebe et al., 2013), higher postoperative analgesic requirements (Aoki et al., 2010), increased pain intensity in irritable bowel syndrome (Pata et al., 2004) and chronic widespread pain (Nicholl et al., 2011). A study investigating the role of HTR2 variants on neuropathic pain relief during escitalopram treatment showed no association of *HTR2A* rs6314 but of *HTR2C* rs6318 with better pain relief (Brasch-Andersen et al., 2011).

In previous studies, we showed an association of SNVs in *TRPV1*, transient receptor potential ankyrin 1 (Binder et al., 2011) and sigma-1 receptor (*SIGMAR1*; Sachau et al., 2019) with changes in thermal and mechanical sensitivity. The contribution of *HTR2A* variants to neuropathic pain susceptibility (ongoing pain) and somatosensory sensitivity profiles (evoked pain) is largely unknown.

Thus, we aimed to compare genotype distribution of SNVs in *HTR2A* between neuropathic pain patients and healthy volunteers. We analysed the impact of *HTR2A* variants on the somatosensory function of neuropathic pain patients assessed by standardized quantitative sensory testing (Binder et al., 2011; Rolke et al., 2006).

2 | METHODS

2.1 | Study populations

A total of 240 patients (mean age 54.52 ± 13.95 years, range 19–84 years; 128 females, 112 males), all white Caucasians, with chronic neuropathic pain syndromes collected within the German Research Network on Neuropathic Pain (DFNS) were included into the study. This study cohort was the same as in previous studies by Binder et al. (2011) and Sachau et al. (2019). The patients suffered from a variety of neuropathic pain syndromes or pain syndromes with neuropathic features, that is, polyneuropathy (PNP; $n = 65$), post-herpetic neuralgia (PHN; $n = 21$), peripheral nerve injury (PNI; $n = 27$), complex regional pain syndrome (CRPS; $n = 68$), trigeminal neuropathy (TGN; $n = 35$), central pain (CP; $n = 11$) and other neuropathies ($n = 13$; Binder et al., 2011). Although CRPS type 1 has recently been defined as nociplastic pain (nerve lesion not demonstratable), CRPS was considered as pain syndrome with neuropathic features in this study as both types, CRPS type I and type II, share similar sensory symptoms and signs (Gierthmühlen et al., 2014). For comparison, 253 healthy German volunteers (mean age 34.12 ± 7.01 years, range 27–67; 172 females, 81 males), all white Caucasians, were collected at the University Hospital Schleswig-Holstein (Gierthmühlen et al., 2015).

All subjects gave their written informed consent. The study was approved by the Ethics Committee of the Medical Faculty of the Kiel University and Ethic Committees of the participating DFNS centres (AZ: 114/05, AZ 131/05, B204/04) and was performed according to the declaration of Helsinki.

2.2 | Phenotypical characterization (quantitative sensory testing)

All patients were phenotypically characterized using standardized quantitative sensory testing (QST), a validated test battery established by the DFNS (www.neuro.med.tu-muenchen.de/dfns/) to determine somatosensory function and symptoms of pain (Magerl et al., 2010; Rolke et al., 2006). Accordance with the protocol was ensured by regular quality control (Vollert et al., 2015, 2016). This QST battery consists of seven tests measuring 13 sensory parameters. Briefly, thermal detection thresholds for the perception of cold (CDT) and warm (WDT), thermal pain thresholds for cold (CPT) and heat (HPT), thermal sensory limen (TSL), paradoxical heat sensation (PHS), mechanical detection thresholds for touch (MDT) and vibration (VDT), mechanical pain threshold (MPT), a stimulus–response function for pinprick sensitivity (MPS) and dynamic mechanical allodynia (DMA), pain summation to repetitive pinprick stimuli (wind-up ratio; WUR) as well as blunt pressure pain threshold (PPT) were assessed in the area of maximum pain and a contralateral mirror image control area. Patient data were included in the study analysis, if at least results of 12 of 13 QST tests were available.

2.3 | Selection of candidate SNVs

Two SNVs in *HTR2A* g.-1438G > A (rs6311) and c.102C > T (rs6313) were selected due to (a) the high frequency of the minor allele, (b) already described associations to various disease stages and (c) potential alteration of transcription (Parsons et al., 2004; Smith et al., 2013).

2.4 | Genotype analysis

Whole-genomic DNA was extracted from venous blood samples obtained from patients and volunteers using the Qiagen Gentra Puregene Blood Kit (Qiagen). The final concentration of genomic DNA was estimated spectrophotometrically (Binder et al., 2011). Genotyping of the selected SNVs was performed by pyrosequencing according to protocols provided by the manufacturer on a PSQ HS96 platform (HS 96 system, Qiagen). For PCR, 1 µl gDNA was added to 25 µl mastermix containing PCR buffer (500 mmol/L KCl, 200 mmol/L Tris-HCl; pH 8.4), 50 mmol/L $MgCl_2$, 10 mmol/L dNTPs (2.5 mmol/L each), forward and reverse primer each 10 µmol/L (one of them biotinylated for pyrosequencing analysis), 75 U Taq polymerase (500 U/µL) and ultra-pure water. All reagents, except the primers (Sigma-Aldrich), were obtained from Thermo Fisher Scientific. For each PCR, two negative controls (NTC) were included. PCR amplification consisted of initial denaturation at 94°C for 4 min, 50 cycles containing 30 s at 94°C, 30 s annealing at 63° and 30 s elongation at 72°C. Final elongation was executed for 7 min at 72°C. Pyrosequencing was conducted as previously described (Bruckmueller et al., 2015). Primer sequences are available on request.

2.5 | Statistical analysis

Statistical analysis was performed by comparing frequencies of selected SNVs between neuropathic pain patients and healthy controls as well as with regard to associations of SNVs to QST parameters in patients.

Z-scores of the respective patient QST values (except for DMA and PHS) were available from a previous study (Binder et al., 2011). Normalization of *z*-scores accounts for differences in sex, age and specific body region, allowing direct comparison with values of healthy controls. *Z*-scores of zero represent the mean of healthy controls, while *z*-scores above '0' indicate a gain of function (hyperalgesia) and *z*-scores below '0' indicate a loss of function (hypoesthesia and hypoalgesia). Abnormal values were determined according to reference data, that is, *z* values below −1.96 were considered as abnormal loss and *z* values above 1.96 as abnormal gain of function (95% confidence interval of relative reference data; Maier et al., 2010; Rolke et al., 2006). DMA and PHS, absent under physiological conditions, were given with original values (DMA: 0–100 numeric rating scale; PHS: numbers of PHS, 0–3). Patients were subdivided into two main clusters following a previous analysis (Binder et al., 2011). These clusters are represented by considerably different somatosensory QST *z*-profiles. Cluster 1 included a group of patients with mainly preserved sensory function, whereas cluster 2 patients were characterized by a relative loss of small and large fibre function.

The two selected genetic variants in *HTR2A* were tested for Hardy–Weinberg equilibrium with chi-squared test and for linkage disequilibrium using Haploview (Barrett et al., 2005). Statistical analysis was performed comparing all three genotypes as well as by use of the dominant genetic model for each SNV comparing wild-type carriers to carriers of at least one variant allele. Allele distribution of selected variants of pain patients was compared to healthy controls using chi-squared test. Minor allele frequencies of patients and controls were compared to published data of the 1000 Genomes Project (1000 Genomes Project Consortium et al., 2015; https://www.ncbi.nlm.nih.gov/snp/) by use of chi-squared test.

To reduce the number of sensory components (QST values) to the principal structure of somatosensory perception in neuropathic pain patients, principal component analysis (PCA) was performed as described elsewhere (Sachau et al., 2019).

Mean values of the QST parameters (*z* values or log transformed for DMA) relevant for each PCA were calculated. Analysis of variance was calculated on these principal somatosensory components (mean values for the parameters describe above) using three-way ANOVAs with the factors 'homozygous wild-type versus heterozygous versus homozygous variant carrier', 'cluster 1 versus cluster 2' and 'male versus female' and post hoc contrasts to identify the potential presence of heterozygous effects. Additional three-way ANOVAs were calculated with the factors 'homozygous wild-type versus variant carriers' (dominant genetic model), 'cluster 1 versus cluster 2' and 'male versus female' to disclose the impact of the variant allele and potential interactions. The influence of the SNVs on each principal component was also calculated for the different patient groups, that is, for all patients, for cluster 1, for cluster 2 and for male or female sex, respectively, using one-way ANOVA. To control for multiple comparison, *p* values were Bonferroni adjusted considering the number of different principal components (resulting in $p < .0125$ for four PCAs and $p < .025$ for a significant trend ($\alpha = 0.10$), see Section 3).

In a second step, the genotype influence of each SNV on single QST parameters (*z*-scores) within the patient cohort was investigated, considering only the QST parameters of the significant principal somatosensory component. One-way ANOVA for the respective QST parameters, sex and variant was performed as described above. In case of significant Levene test for equality of variances (<0.05), Welch's *t* test was used. Differences of DMA and PHS were calculated with Kruskal–Wallis test. In case of significant differences in *z* values, a gene-dose effect for the three genotypes was calculated using a linear regression model.

Differences in frequencies of abnormal QST values were assessed with chi-squared test and Fisher's exact test for $n < 5$. For this calculation, QST values were encoded as nominal values with three categories (within normal range; abnormal loss; abnormal gain of somatosensory function). PHS and DMA were encoded as dichotomized variables (absent = normal; present = abnormal). Differences were analysed (a) within the entire patient cohort and (b) separately within cluster 1 and 2 patients. Analysis was also performed for male and female patients separately.

To control for multiple comparison, *p* values were Bonferroni adjusted considering the number of parameters within the significant principal component (resulting in $p < .0167$ for the three parameters of PC2 and $p < .033$ for a significant trend ($\alpha = 0.10$), see Section 3).

Finally, a modified three-cluster analysis was used to subgroup patients based on their QST profiles as described more recently (Baron et al., 2017). These clusters have been shown to be similar to mechanistic surrogate models of neuropathy (Vollert et al., 2018). Cluster SL was characterized by a sensory loss (loss of small and large fibre function with PHS), cluster TH showed a thermal hyperalgesia (preserved sensory function in combination with heat and cold hyperalgesia and mild DMA) and cluster MH showed a mechanical hyperalgesia (loss of small fibre function with pinprick hyperalgesia and DMA). The frequency of each genotype in the three clusters was calculated by use of the chi-squared test. Due to the small sample size, calculation of the genotype influence of

TABLE 1 Demographic data of patients

	All neuropathic pain patients	Cluster 1	Cluster 2	Cluster SL	Cluster TH	Cluster MH
N	234[a]	117	117	33	120	81
Age (years) Range	54.67 ± 13.98 19–84	52.32 ± 13.63 19–81	57.07 ± 13.99 24–84	54.8 ± 14.46 30–81	53.67 ± 13.53 19–81	56.11 ± 14.50 24–84
Females (%)	52.6	55.6	49.6	39.4	61 7	44.4
Males (%)	47.4	44.4	50.4	60.6	38.3	55.6
Disease duration (months)	37.87 ± 63.96	40.26 ± 79.31	35.82 ± 47.43	53.60 ± 54.54	32.22 ± 56.05	38.22 ± 78.45
Mean pain intensity (0–10 Likert-Scale NRS)	5.90 ± 2.19	5.71 ± 2.09	6.11 ± 2.27	5.54 ± 2.0)	5.71 ± 2.27	6.31 ± 2.10
Pain group (*n*, %)						
Moderate	117 (50.0)	65 (55.6)	52 (44.4)	17 (51.5)	62 (51.7)	38 (46.9)
Severe	77 (32.9)	34 (29.1)	43 (36.8)	7 (21.2)	38 (31.7)	32 (39.5)
Missing data	40 (17.1)	18 (15.4)	22 (18.8)	9 (27.3)	20 (16.7)	11 (13.6)
Polyneuropathy (*n*, %)	65 (27.8)	24 (20.5)	40 (34.2)	18 (54.4)	26 (21.7)	20 (24.7)
Post-herpetic neuralgia (*n*, %)	20 (8.5)	6 (5.1)	14 (12.0)	4 (12.1)	7 (5.8)	9 (11.1)
Peripheral nerve injury (*n*, %)	27 (11.5)	13 (11.1)	14 (12.0)	4 (12.1)	14 (11.7)	9 (11.1)
Complex regional pain syndrome (*n*, %)	65 (27.8)	38 (32.5)	27 (23.1)	4 (12.1)	43 (35.8)	18 (22.2)
Trigeminal neuropathy (*n*, %)	34 (14.5)	27 (23.1)	7 (6.0)	0	22 (18.3)	12 (14.8)
Central pain (*n*, %)	11 (4.7)	4 (3.4)	7 (6.0)	2 (6.1)	2 (1.7)	7 (8.6)
Other neuropathy (*n*, %)	13 (5.6)	5 (4.3)	8 (6.8)	1 (3.0)	6 (5.0)	6 (7.4)

Note: Values are given as mean ± single standard deviation, percentages or absolute values, respectively. Mean pain intensity was assessed using a numeric rating scale (NRS; 0 = no pain, 10 = worst pain imaginable).

Abbreviations: MH, mechanical hyperalgesia; SL, sensory loss; TH, thermal hyperalgesia.

[a]Genotyping of six DNA samples was not possible.

each SNV on the PCs and single QST parameters (see above) was not performed for the three clusters.

Finally, the same models of analysis described above were also used to analyse the magnitude of ongoing pain. To test whether an association of the variant allele with the relevant principal somatosensory component predicts the magnitude of ongoing pain was tackled with additional analysis using chi square and correlation analysis.

All statistical evaluations were performed using statistical software SPSS 23.0 (IBM).

3 | RESULTS

Due to technical issues, that is, no clear genotyping results, six patients were excluded from further analysis. Of the remaining 234 patients, 117 were characterized by mainly preserved somatosensory function (cluster 1 according to Binder et al., 2011) and 117 by relative loss of small and large fibre function (cluster 2). According to the modified three-cluster analysis (Baron et al., 2017), 120 patients were characterized by preserved function and thermal hyperalgesia (cluster TH), which mostly contained the cluster 1 cases ($n = 92$). In all, 81 patients were characterized by sensory loss with mechanical hyperalgesia (cluster MH) and 33 patients by sensory loss without hyperalgesia (cluster SL), each mostly containing cluster 2 cases ($n = 61$ and $n = 28$, respectively). Patients characteristics are shown in Table 1 and comparison of two-cluster versus three-cluster solution in Figure 1.

3.1 | HTR2A genotype distribution between patients and controls

Due to poor genotyping results, 5 healthy controls were excluded for *HTR2A* rs6311 and 14 for rs6313. Frequencies

of the *HTR2A* rs6311 and rs6313 were in Hardy–Weinberg equilibrium for patients ($p = .737$ and $p = .308$) and controls ($p = .758$ and $p = .831$).

As *HTR2A* rs6311 and rs6313 genetic variants were almost in complete linkage disequilibrium ($r^2 = 0.97$, $D' = 0.99$), results of statistical analysis are only described in detail for rs6313. There were no significant differences in genotype distribution of the investigated SNVs between neuropathic pain patients, healthy controls and published data from 1000 Genome Project (1000 Genomes Project Consortium et al., 2015; Sherry et al., 2001; Table 2).

3.2 | The somatosensory profile of neuropathic pain patients

To investigate possible effects of SNVs in *HTR2A* on the somatosensory function of neuropathic pain patients, we analysed the association between the selected SNVs and the standardized QST values of patients using a stepwise approach analysing (a) the effect on principal components of the QST parameters and (b) the effect on relevant single QST parameters.

3.3 | Principal component analysis

Principal component analysis revealed four major independent somatosensory components (Table 3) with the first principal component (PC1, 21.05% of total variance) defined by thermal detection parameters (CDT, WDT, TSL) and some factor loading of heat pain (HPT). The second most important principal component (PC2; 14.53% of total variance) was constituted by punctate mechanical nociception (MPT, MPS) as well as pain to light touch (DMA) and a smaller negative factor loading of temporal summation (WUR). PC3 (13.13% of total variance) comprised thermal and blunt pressure nociception, as well as pain summation (CPT, HPT, PPT, WUR) and PC4 (10.53% of total variance) was characterized by mechanical detection parameters (MDT, VDT). As WUR was part of two principal components (PC2 and PC3), it was excluded for further calculation as being inconclusive and likely to blur selectivity. Due to the physiological meaning of the different components, HPT was included in PC3 (thermal/pressure nociception) rather than PC1 (thermal detection). As shown previously, the shared variance of WDT and HPT relates to both sets of primary afferents expressing the TRPV1 receptor (Yarmolinsky et al., 2016). PC2 will be interpreted as reflecting CS as it comprises sensitivity to punctate stimuli (MPT, MPS) as well as pain to light touch (DMA).

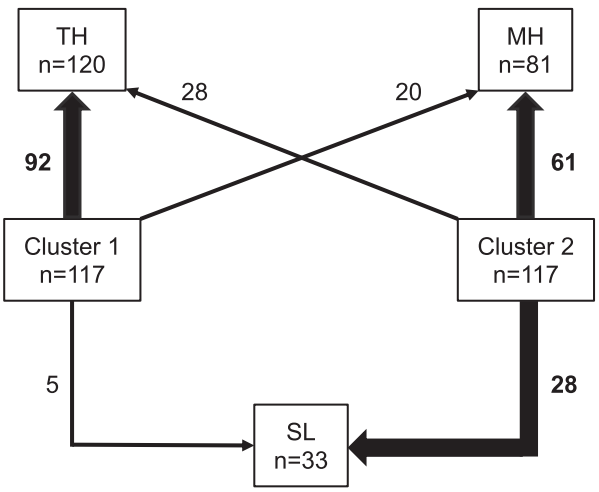

FIGURE 1 Allocation of the two-cluster to the three-cluster approach. The thermal hyperalgesia cluster (TH) mainly consists of cluster 1 patients (preserved sensory function). In contrast, the mechanical hyperalgesia cluster (MH) and the sensory loss cluster (SL) mainly consist of cluster 2 patients (sensory loss)

Applying the dominant genetic model, both ANOVAs on PC1 (thermal detection) and on PC3 (thermal/pressure nociception) revealed a strong effect of cluster 1 versus 2 ($p < .0001$) and of sex ($p = .009$, $p < .0001$, respectively) as well as a significant cluster × sex interaction ($p = .001$, $p = .002$). ANOVA on PC4 (mechanical detection) revealed only a strong effect of cluster 1 versus 2 ($p < .001$).

Only for PC2 (MPT, MPS, DMA = mechanical hyperalgesia/CS), three-way ANOVA revealed a significant trend towards an impact of the rs6313 genotype ($F_{2,222} = 4.121$, $p = .017$). Post hoc contrasts revealed only a marginally difference in the magnitude of PC2 comparing homozygous variant carriers to homozygous wild-type carriers ($+0.35 \pm 0.15$ vs. -0.11 ± 0.11, $p = .052$). There was no effect of cluster or sex, and no other significant interactions on PC2.

A closer look on PC2 magnitude revealed that the magnitude in heterozygous or homozygous variant allele carriers was almost identical ($+0.22 \pm 0.10$ vs. $+0.35$ vs. 0.16; Figure 2). We thus decided to apply the dominant genetic model, that is, comparing homozygous wild-type carriers and carriers of at least one variant allele. This approach disclosed a significant impact of rs6313 on PC2 ($F_{1,226} = 7.585$, $p = .006$), but only a weak and non-significant impact of cluster 1 versus 2 ($F_{1,226} = 2.172$, $p = .142$), and no impact of male versus female sex ($F_{1,226} = 0.049$, $p = .825$). Notably, there was a cluster × genotype interaction on PC2 ($F_{1,226} = 4.478$, $p = .035$, no longer significant after Bonferroni adjustment). In cluster 2 patients (relative loss of small and large fibre function), z values of wild-type carriers differed significantly from variant allele carriers (-0.34 ± 0.15 vs. $+0.31 \pm 0.11$ vs., $p < .001$). In contrast, the difference in cluster 1 patients (mainly preserved function) was much smaller and did not reach significance ($+0.13 \pm 0.15$ vs. $+0.20 \pm 0.12$, $p > .50$). Results

TABLE 2 Minor allele frequencies (MAF) of each SNV in *HTR2A* in healthy controls, neuropathic pain patients and according to 1000 Genomes

Single nucleotide variant	MAF in healthy controls	MAF in neuropathic pain patients	MAF according to 1000 Genomes[a]
c.102C > T (rs6313)	0.41	0.41	0.44
g.−1438G > A (rs6311)	0.41	0.42	0.44

Note: There were no significant differences in genotype distribution between neuropathic pain patients, healthy controls and published data from 1000 Genome Project (1000 Genomes Project Consortium et al., 2015) assessed with chi-squared test.

Abbreviation: MAF, minor allele frequency.

[a] Allele frequencies in the European subpopulation according to the 1000 Genome Project (state 07/2019; https://www.ncbi.nlm.nih.gov/snp/).

TABLE 3 Loading values of the 13 QST parameters included in the principal component analysis (PCA) for the four principal sensory components (PC1-4)

QST parameters	PC1	PC2	PC3	PC4
CDT	**0.807**[a]	0.046	0.090	0.086
WDT	**0.837**[a]	−0.082	0.109	0.035
TSL	**0.904**[a]	−0.057	0.037	0.087
CPT	0.238	0.044	**0.666**[a]	0.030
HPT	**0.539**[a]	0.135	0.497	0.062
PPT	0.022	0.214	**0.691**[a]	0.124
MPT	−0.016	**0.741**[a]	0.093	0.203
MPS	0.045	**0.841**[a]	0.220	0.095
WUR	−0.068	**−0.566**[a]	**0.534**[a]	−0.032
MDT	0.300	0.137	0.015	**0.687**[a]
VDT	−0.034	0.098	0.129	**0.782**[a]
DMA	−0.130	0.450	0.398	−0.371
PHS	−0.322	−0.059	−0.001	−0.247
% total variance	21.05	14.53	13.13	10.53
Interpretation	Thermal detection	Mechanical hyperalgesia	Thermal/pressure hyperalgesia	Mechanical detection

Note: Factorial structure of QST data (after factor rotation using normalized VARIMAX).

[a]Factors exceeding factor loadings of ±0.50.

of three-way ANOVAs of rs6313 genotype, clusters and sex as well as one-way ANOVAs for the different patient groups (all patients, cluster 1, cluster 2) on PC1-4 are shown in Table 4.

In summary, the PCA analysis revealed no significant impact of genotype on thermal detection, thermal/pressure hyperalgesia and mechanical detection; thus, the effect of genotype was specific for mechanical nociception.

3.4 | Effects of HTR2A rs6313 on single QST parameters

In the next step, the association of the rs6313 genotype to the somatosensory profile (*z*-scores or nominal values of the 13 QST parameters) was investigated focusing on the single QST parameters of the relevant PC2, that is, MPS, MPT and DMA (Tables 5 and 6). In the entire patient cohort, patients with at least one variant allele exhibited an increased sensitivity to mechanical pain (MPS, +0.70 ± 0.11 vs. +0.28 ± 0.17, $p = .036$; MPT, +0.57 ± 0.13 vs. +0.11 ± 0.16, $p = .032$, no longer significant after Bonferroni adjustment) compared to wild-type carriers (Table 5). Differences became more distinct within the group of cluster 2 patients (sensory loss) with two groups of patients, that is, with and without accompanying mechanical hyperalgesia (MPS, $p = .009$; MPT, $p = .002$). The binary analysis of patients with abnormal sensory response (*z*-scores above 1.96 or below −1.96) revealed that variant carriers in cluster 2 exhibited less frequently abnormal decreased sensitivity to mechanical pain compared to wild-type carriers (4% vs. 20%; $p = .016$; Table 6). For DMA, no significant difference was observed neither in the entire patient cohort nor in cluster 2. In cluster 1, there was no significant association between rs6313 genetic variant and a

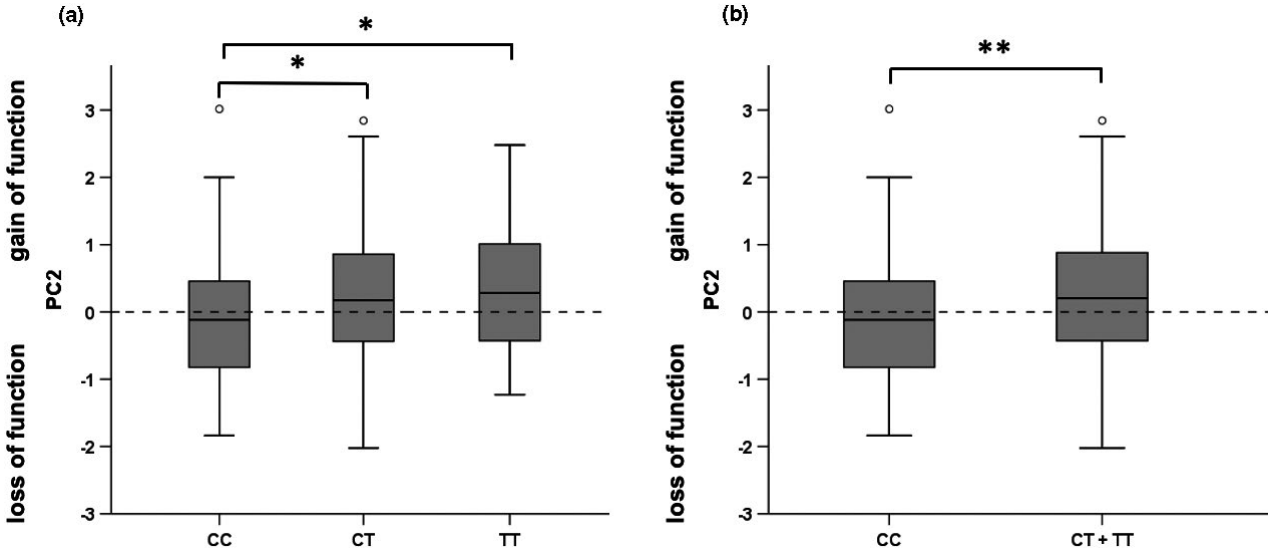

FIGURE 2 Effect of HTR2A rs6313 on the mechanical hyperalgesia component PC2 (MPT, MPS and DMA). (a) Heterozygous carriers and homozygous variant carriers were characterized by similar *z* values for PC2 with no significant differences overall. (b) In contrast, there was a significant difference when applying the dominant genetic model, that is, comparing homozygous wild-type carriers (CC) and carriers of at least one variant allele (CT + TT; $p = .008$). One-way ANOVA was performed, uncorrected *p* value: $*p < .05$, $**p < .01$

QST parameter of PC2. The somatosensory profile of the entire patient cohort, cluster 1 and cluster 2 is shown in Figure 3.

3.5 | Gene-dosing effect of HTR2A rs6313

As there was an effect of HTR2A rs6313 on the mechanical hyperalgesia component in particular in patients with a sensory loss, PC2 and the respective single QST parameters (MPT, MPS and DMA) were assessed for a gene-dose effect, defined as association of the number of variant alleles with z-scores, in cluster 2 (Figure 4). There was a significant gene-dose effect regarding PC2 ($\beta = 0.362$, $p = .004$) and MPT with an increase in MPT *z*-scores of $\beta = 0.648$ from wild-type to heterozygous carriers and from heterozygous to variant carriers ($p = .001$). While homozygous cluster 2 variant carriers exhibited higher positive MPT *z*-scores than heterozygous carriers indicating hypersensitivity, wild-type carriers were characterized by negative *z*-scores indicating mechanical hyposensitivity. However, when looking at MPS and DMA only, there was no evidence for a gene-dose effect. Thus, the expression varied to either side for heterozygous versus homozygous variant carriers in the three QST parameters suggesting that a dominant genetic effect of the variant allele rather than gene-dosing describes the differences best.

3.6 | Sex-dependent analysis

To detect gender-dependent differences, statistical analysis was performed for male and female patients separately in a next step (data not shown). Genotype-associated somatosensory differences in male patients were similar to those of the entire patient cohort. In male patients, the variant allele was associated with an increased sensitivity to mechanical pain compared to the wild-type (MPT, $+0.84 \pm 0.20$ vs. $+0.09 \pm 0.24$, $p = .028$). In cluster 2, variant carriers also exhibited a trend towards an increased sensitivity to mechanical pain stimuli (MPT, $+0.76 \pm 0.27$ vs. -0.46 ± 0.39, $p = .015$; MPS, $+0.66 \pm 0.23$ vs. -0.29 ± 0.28, $p = .024$) as well as less frequently abnormal decreased sensitivity to mechanical pain compared to wild-type carriers (5% vs. 29%, $p = .017$). By contrast, there were no significant differences regarding female patients alone.

3.7 | Frequency of HTR2A rs6313 across three clusters

Although the current analysis was based on two distinct patient groups used in our previous genetic studies (Binder et al., 2011; Sachau et al., 2019), we reassessed our findings with respect to the recently published three-cluster solution (Baron et al., 2017) that had been validated in mechanism-based human surrogate models (Vollert et al., 2018).

Differences in the genotype frequency (wild-type, heterozygous, variant) were also calculated applying the recently published three-cluster solution (Table 7). In that cluster analysis, patients with sensory loss (cluster 2) are subdivided mainly into those with sensory loss only (SL) and those with additional mechanical hyperalgesia (MH; Figure 1). Consistent with the PCA shown above, the frequency of homozygous variant carriers was higher in MH patients than in SL patients (51% vs. 17%). In contrast, frequency of homozygous wild-type carriers was highest in patients characterized

TABLE 4 ANOVA of c.102C > T, clusters and sex on PC1-4

	PC1		PC2		PC3		PC4	
	CDT, WDT, TSL		MPT, MPS, DMA		CPT, HPT, PPT		MDT, VDT	
	CC versus CT versus TT	CC versus CT + TT	CC versus CT versus TT	CC versus CT + TT	CC versus CT versus TT	CC versus CT + TT	CC versus CT versus TT	CC versus CT + TT
Three-way ANOVA								
1: c.102C > T (rs6313)	0.475 (0.623)	0.045 (0.832)	**4.121 (0.017(*))**	**7.585 (0.006*)**	0.183 (0.833)	0.270 (0.604)	0.640 (0.528)	0.538 (0.464)
2: cluster 1 versus 2	**65.489 (<0.0001*)**	**71.492 (<0.0001*)**	1.174 (0.280)	2.172 (0.142)	**17.609 (<0.0001*)**	**17.344 (<0.0001*)**	**69.831 (<0.0001*)**	**80.026 (<0.0001*)**
3: sex male versus female	3.272 (0.072)	**6.945 (0.009*)**	0.165 (0.685)	0.049 (0.825)	**20.195 (<0.0001*)**	**24.412 (<0.0001*)**	2.462 (0.118)	2.093 (0.149)
1 × 2 interaction	0.594 (0.553)	1.089 (0.298)	2.374 (0.095)	**4.478 (0.035)**	0.882 (0.416)	0.623 (0.431)	0.607 (0.546)	0.683 (0.410)
1 × 3 interaction	2.000 (0.138)	0.542 (0.470)	0.713 (0.491)	0.964 (0.327)	0.386 (0.680)	0.113 (0.737)	0.222 (0.801)	0.490 (0.485)
2 × 3 interaction	**4.290 (0.039)**	**11.085 (0.001*)**	0.619 (0.432)	0.203 (0.653)	**6.555 (0.011*)**	**9.661 (0.002*)**	1.251 (0.265)	3.331 (0.069)
1 × 2 × 3 interaction	1.779 (0.171)	0.906 (0.342)	0.209 (0.811)	0.051 (0.821)	1.453 (0.236)	2.601 (0.108)	1.523 (0.220)	2.981 (0.086)
One-way ANOVA c.102C > T								
All patients	1.974 (0.141)	0.256 (0.613)	**3.813 (0.023(*))**	**7.147 (0.008*)**	0.076 (0.892)	0.066 (0.797)	1.593 (0.206)	0.323 (0.570)
Cluster 1	1.336 (0.267)	1.195 (0.277)	0.481 (0.619)	0.136 (0.713)	0.343 (0.710)	0.180 (0.672)	0.014 (0.986)	0.005 (0.945)
Cluster 2	1.195 (0.277)	0.203 (0.817)	**5.826 (0.004*)**	**11.753 (0.001*)**	0.196 (0.822)	0.004 (0.948)	0.762 (0.469)	0.433 (0.512)

Note: F value (uncorrected $p < .05$ are marked in bold black; *significant after Bonferroni adjustment, (*)significant trend after Bonferroni adjustment.

TABLE 5 Associations of *HTR2A* c.102C > T (rs6313) with somatosensory function reflected by QST parameters of the relevant PC2

		MPT		MPS		DMAlog	
Genotype	***N***	***z*-score**	***p* value[a]**	***z*-score**	***p* value[a]**	**Mean**	***p* value[b]**
All patients							
CC	81	0.11 ± 0.16 (1.43)	**.032**$^{(*)}$	0.28 ± 0.17 (1.50)	**.036**	−0.70 ± 0.08 (0.68)	.174
CT + TT	153	0.57 ± 0.13 (1.62)		0.70 ± 0.11 (1.42)		−0.50 ± 0.07 (0.84)	
Cluster 1							
CC	40	0.56 ± 0.19 (1.22)	.838	0.65 ± 0.23 (1.48)	.766	−0.81 ± 0.09 (0.57)	.301
CT + TT	77	0.50 ± 0.19 (1.68)		0.73 ± 0.16 (1.39)		−0.62 ± 0.08 (0.72)	
Cluster 2							
CC	41	−0.34 ± 0.23 (1.50)	**.002***	−0.09 ± 0.23 (1.45)	**.009***	−0.60 ± 0.12 (0.76)	.329
CT + TT	76	0.64 ± 0.18 (1.56)		0.66 ± 0.17 (1.45)		−0.37 ± 0.11 (0.94)	

Note: *Z*-scores are given as arithmetic mean ± standard error of the mean (standard deviation).

Uncorrected $p < .05$ are marked in bold black, *significant after Bonferroni adjustment; $^{(*)}$significant trend after Bonferroni adjustment.

Abbreviations: DMA, dynamic mechanical allodynia; MPS, mechanical pain sensation; MPT, mechanical pain threshold.

[a]*p* values for analysis of variance (ANOVA).

[b]*p* values for Kruskal–Wallis test.

TABLE 6 Associations of *HTR2A* c.102C > T (rs6313) with frequencies of abnormal QST parameters of the relevant PC2

		MPT				MPS				DMA	
		Loss		Gain		Loss		Gain		Gain	
Genotype	***N***	***n* (%)**	***p* value**	***n* (%)**	***p* value**	***n* (%)**	***p* value**	***n* (%)**	***p* value**	***n* (%)**	***p* value**
All patients											
CC	81	8 (9)	.180	9 (11)	.097	1 (1)	1.000	10 (12)	.240	21 (26)	.285
CT + TT	153	8 (5)		30 (20)		1 (0.7)		28 (18)		50 (33)	
Cluster 1											
CC	40	0 (0)	.164	6 (15)	.549	0 (0)	1.000	5 (13)	.533	7 (18)	.376
CT + TT	77	5 (6)		15 (19)		1 (1)		13 (17)		19 25)	
Cluster 2											
CT + TT	76	3 (4)	**0.016***	15 (20)	0.107	0 (0)	0.350	15 (20)	0.301	31 (41)	0.481

Note: *p* values for chi-squared test or Fisher's exact test for $n < 5$.

Uncorrected $p < .05$ are marked in bold black, *significant after Bonferroni adjustment.

Abbreviations: DMA, dynamic mechanical allodynia; MPS, mechanical pain sensation; MPT, mechanical pain threshold.

by thermal hyperalgesia (cluster TH) and lowest in patients with sensory loss (cluster SL, 53% vs. 21%).

3.8 | An interaction of HTR2A rs6313 genotype with the QST phenotype predicts levels of ongoing pain

The majority of patients (194/234) provided estimates of their levels of ongoing pain. They were analysed with the same three-way model of ANOVA that we had used for the analysis of the expression of the PC2 component of QST (Table 8). The three-way ANOVA explaining a total of 5.8% of variance revealed a complete absence of an overall difference in pain levels between clusters 1 and 2 ($F_{1,186} = 0.007$, $p = .935$) in line with the absence of a significant difference in pain between patients of cluster 1 and 2 (5.71 ± 0.21 vs. 6.11 ± 0.21, $p > .20$). Moreover, there was a weak but non-significant impact of sex and genotype ($F_{1,186} = 3.203$, $p = .075$ and $F_{1,186} = 3.203$, $p = .135$; respectively). However,

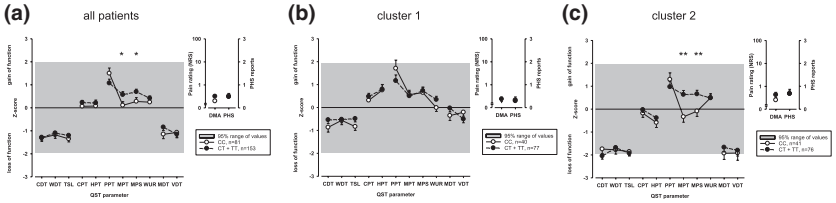

FIGURE 3 Influence of HTR2A rs6313 on the somatosensory profile. (a) Somatosensory profile in the entire cohort of patients indicated that variant carriers (CT + TT; $n = 153$) exhibit an increased pinprick sensitivity compared to wild-type carriers (CC; $n = 81$), depicted by both threshold (MPT) and suprathreshold assessment (MPS). (b) In cluster 1, there was no significant association between *HTR2A* rs6313 genetic variant and a QST parameter. (c) In contrast, differences became more distinct when focusing on cluster 2 with significant differences for both MPT and MPS. For the somatosensory profile analysis of variance (ANOVA) and for dichotomized QST parameters (PHS, DMA), Kruskal–Wallis test were performed, uncorrected p values: $^*p < .05$; $^{**}p < .01$. Values are given as arithmetic mean and standard error of the mean. NRS, numeric rating scale; CDT, cold detection threshold; WDT, warm detection threshold; TSL, thermal sensory limen; CPT, cold pain threshold; HPT, heat pain threshold; PPT, pressure pain threshold; MPT, mechanical pain threshold; MPS, mechanical pain sensitivity; WUR, wind-up ratio; MDT, mechanical detection threshold; VDT, vibration detection threshold; DMA, dynamic mechanical allodynia; PHS, paradoxical heat sensation

there was a strong impact of a cluster × genotype interaction ($F_{1,186} = 11.640$, $p \leq .001$), but no other significant interactions (all $p > .30$, Table 8). Post hoc analysis revealed that pain levels were not different between wild-type and variant allele carriers in cluster 1 (6.11 ± 2.02 vs. 5.51 ± 2.12, $p > .05$). In contrast, ongoing pain in cluster 2 patients being variant allele carriers was >30% higher than in wild-type carriers (6.59 ± 2.32 vs. 5.05 ± 1.79, $p < .001$).

Since this pattern replicated the expression of the PC2 QST component, we tested whether ongoing pain would be different if patients stratified for gain or loss in the PC2 component. Patients with PC2 z values > 0 (PC2 gain, $n = 104$) presented with significantly higher pain ratings than patients with PC2 z values < 0 (PC2 loss, $n = 90$; 6.21 ± 0.20 vs. 5.55 ± 0.24, $p < .05$). The PC2 QST component encompassed hyperalgesia to punctate stimuli (MPT and MPS) and pain to light touch (DMA) and is the main constituent of the mechanical hyperalgesia phenotype in the three-cluster approach (cluster MH). Most of these patients were members of cluster 2 (Figure 1). Indeed, patients of the mechanical hyperalgesia phenotype presented with significantly higher pain than patients of both other phenotypes, that is, sensory loss or thermal hyperalgesia (5.67 ± 0.20 vs. 6.31 ± 0.25, $p < .05$). Additionally, the magnitude of the PC2 z value was significantly correlated with the magnitude of ongoing pain ($r = 0.19$, $p < .01$). Moreover, the PC2 gain phenotype was significantly more frequent in the variant allele carriers (87/153 = 57%) than in the CC wild-type carriers (33/81 = 41%) as tested by chi square ($\chi^2 = 5.51$, $p < .02$).

4 | DISCUSSION

The present study aimed at elaborating the influence of *HTR2A* genetic variants on the extent of sensory abnormalities and ongoing pain intensity in patients with neuropathic pain. The rs6313 variant was associated with enhanced pinprick perception in neuropathic pain patients, in particular in subjects with a relative loss of small and large fibre function. These patterns were documented using principal somatosensory components, the relevant QST parameters constituting this component, and two different approaches of cluster analyses: two-cluster (Binder et al., 2011) versus three-cluster solution (Baron et al., 2017).

Previous studies have shown an involvement of HTR in nociceptive processing operating via spinal cord modulating facilitatory or inhibitory descending pathways. Of the seven known HTR subfamilies, HTR2 is thought to play an important role in spinal pain modulation. The HTR2A subtype is widely expressed in peripheral tissues, by dorsal root ganglion cells and primary afferent fibres but also in the central nervous system (Van Steenwinckel et al., 2009). Previous studies described a predominantly facilitatory role for HTR2A in acute nociceptive, inflammatory and neuropathic pain states via downstream activation of phospholipase C (Millan, 2002). In the spinal nerve injury model, serotonergic descending pathways modulate PKCγ spinal interneurons through HTR2A activation leading to mechanical hyperalgesia (Artola et al., 2020), which is associated with spinal morphological reorganization (Alba-Delgado et al., 2018). In an incision model, HTR2A mediated c-fos expression (Silveira et al., 2010) and downregulation of the chloride transporter KCC2 leading to a sensitizing shift of the anion equilibrium potential (Dong et al., 2016). While HTR2A agonists contribute to orofacial inflammatory hyperalgesia (Xue et al., 2020), selective HTR2A blockers and inhibitors of the HTR2A/PSD95-binding complex are antihyperalgesic in neuropathic pain models (Aira et al., 2010; Rahman et al., 2011; Vogrig et al., 2013; Wang et al., 2012; Wattiez et al., 2017). However, an association of *HTR2A* genetic variants with neuropathic pain is largely unknown.

Our analysis showed no significant differences in the distribution of genetic variants between patients and healthy

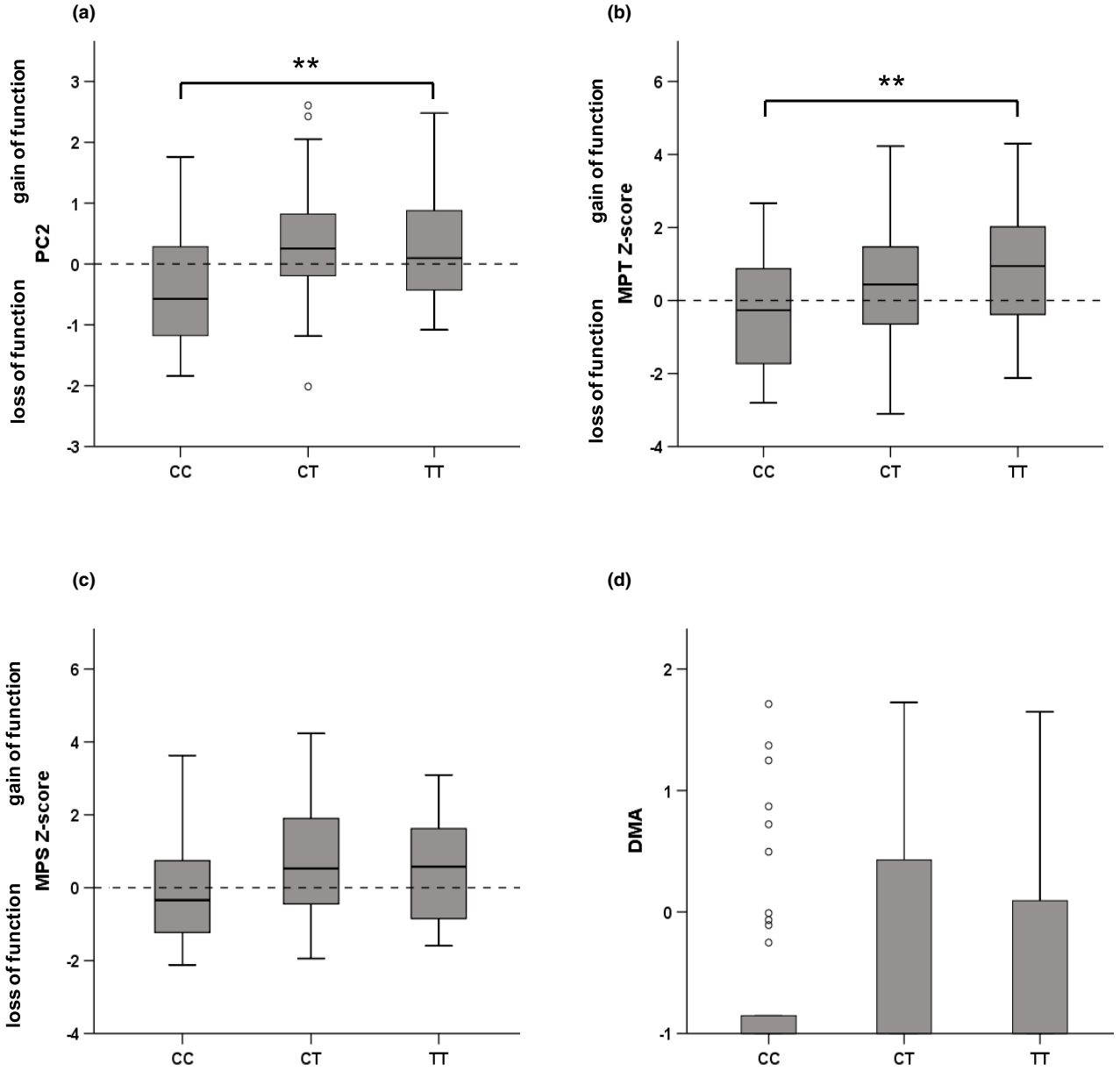

FIGURE 4 Gene-dose effect of HTR2A rs6313 in cluster 2 patients. (a) Comparing all three genotypes, a gene-dose effect could be seen on PC2 ($p = .004$). (b) This effect was also shown for MPT alone ($p = .001$). While homozygous cluster 2 variant carriers (TT; $n = 25$) exhibited higher positive *z*-scores than heterozygous carriers (CT; $n = 51$) and wild-type carriers (CC; $n = 41$) exhibiting a negative z-score indicating mechanical hyposensitivity. (c and d) In contrast, there was no evidence for a gene-dose effect for MPS and DMA alone. Linear regression analysis was performed to calculate for a gene-dose effect; uncorrected *p* values: $^{**}p < .01$

TABLE 7 Frequency of *HTR2A* c.102C > T (rs6313) across the three clusters

Genotype	Cluster SL (sensory loss)	Cluster TH (thermal hyperalgesia)	Cluster MH (mechanical hyperalgesia)	Number of patients
CC	17 (21%)	43 (53%)	21 (26%)	81
CT	9 (8%)	64 (57%)	39 (35%)	112
TT	7 (17%)	13 (32%)	21 (51%)	41

Note: Absolute number and frequency (in round brackets) of each phenotype in wild-type, heterozygous and variant rs6313 carriers.

TABLE 8 ANOVA of c.102C > T, clusters and sex on mean pain intensity

	df (model, error)	*F* value	*p* value
1: c.102C > T (rs6313) CC versus CT + TT	1.186	2.253	.135
2: cluster 1 versus 2	1.186	0.007	.935
3: sex male versus female	1.186	3.203	.075
1 × 2 interaction	1.186	11.640	**.001**[*]
1 × 3 interaction	1.186	0.811	.369
2 × 3 interaction	1.186	0.098	.755
1 × 2 × 3 interaction	1.186	0.022	.883

[a]*significant $p < .05$ are marked in bold black.

controls. The rs6313 variant was associated with differences in somatosensory function in neuropathic pain patients in a pathway-specific manner. Selective modulation of somatosensory processing by genetic variants was recently described for genes coding SIGMAR1, TRP channels, μ-opioid receptor and cyclooxygenase 1 and 2 (Binder et al., 2011; Lee et al., 2006; Sachau et al., 2019).

In our study, the rs6313 variant was associated with the mechanical hyperalgesia principal component and respective single QST parameters. Variant carriers exhibited increased pinprick hyperalgesia than wild-type carriers, depicted by threshold and suprathreshold assessments. Mechanical pinprick hyperalgesia and dynamic mechanical allodynia are somatosensory hallmark signs of CS (Baron et al., 2012; Treede, 2016; von Hehn et al., 2012; Woolf, 2011). This phenomenon develops as a consequence of peripheral hyperactivity leading to an increase in general excitability of nociceptive and multi-receptive spinal cord dorsal horn projection neurons (wide-dynamic-range neurons; Baron et al., 2010). If CS is established, tactile stimuli become capable of activating spinal cord pain signalling neurons via Aβ-mechanoreceptors leading to pain to stroking light touch (dynamic mechanical allodynia) and enhanced activation by Aδ-mechanoreceptors leading to pinprick hyperalgesia (Henrich et al., 2015; LaMotte et al., 1991; Magerl et al., 2001; Simone et al., 1991; Ziegler et al., 1999). CS is modulated by descending systems from brainstem centres (Millan, 2002). Animal models suggest that tonic activation of descending serotonergic pathways is involved in CS in neuropathic pain (Ossipov et al., 2010; Suzuki & Dickenson, 2005). In particular, HTR2A is thought to have a pro-nociceptive function on spinal nociceptive transmission and CS (Aira et al., 2010; Rahman et al., 2011; Vogrig et al., 2013; Wang et al., 2012; Wattiez et al., 2017). These observations may explain the differential effect of rs6313 on pinprick hyperalgesia as surrogate of CS. The rs6313 variant or variants closely linked to this synonymous variant might lead to a functional gain of HTR2A and could be considered as a relevant genotype facilitating CS.

These assumptions are supported by a study investigating the effect of SNVs in different genes on chronic pelvic pain, that is, interstitial cystitis (Cassão et al., 2019). Although no significant associations with disease prevalence or pain severity were observed for rs6313, the rs6311 variant allele was associated with severe pain (results were not corrected for multiple testing). In our study, the promoter variant rs6311 was in almost complete linkage disequilibrium to rs6313. Thus, genetic association of rs6313 is often attributed to functional consequences of rs6311 (Ruble et al., 2016). The rs6311 SNV is localized in the upstream promoter region of HTR2A and might influence transcription. It has been reported that the rs6311 variant increases *HTR2A* promoter activity via binding of specific transcription factors (Parsons et al., 2004). Thus, it might lead to an increased *HTR2A* expression in sensory neurons and facilitate pain generation. Other studies suggest a contradictory role for rs6311 (Myers et al., 2007), for example, a diminished protein expression by reduced extended 5'-UTR formation (Smith et al., 2013). The rs6313 genetic variant is a synonymous SNV localized in exon 1. Although no functional role has been described so far, rs6313 might influence RNA processing, that is, alternative splicing by affecting binding of splicing factors (Ruble et al., 2016). Nevertheless, functional studies are needed to clarify the molecular consequences of rs6311 and rs6313.

The principal component analysis revealed a significant impact of rs6313 on mechanical nociception (PC2) in sensory loss patients (cluster 2). Accordingly, in cluster 2, the observed somatosensory differences for rs6313 became more distinct and the hyperalgesia could be considered as functional rescue from sensory loss, which was seen in homozygous wild-type carriers. In contrast, patients with mainly preserved sensory function (cluster 1) showed no significant association of rs6313 to any QST parameter. CS is influenced by hyperactivity in peripheral nociceptors and descending modulatory effects on spinal cord neurons (Baron et al., 2013). Thus, a differential effect of *HTR2A* variants on pinprick hyperalgesia might be more obvious in patients who are lacking the modulatory effect of peripheral activity (cluster 2).

Sex-dependent genetic influence on pain perception and psychiatric phenotypes is well known (Greenspan et al., 2007; Lebe et al., 2013). Lebe et al. identified a gender-dependent modulatory effect of rs6311 on depression and physical function in pain after lumbar disc surgery (Lebe et al., 2013). The variant allele was associated with higher depression scores in female patients confronted with pain but not in males. In our study, no relevant impact of gender has been identified in three-way ANOVA on PC2. Thus, observed differences between male and female patients are more likely linked to allocation of underlying diseases.

Applying a more refined stratification by the modified three-cluster analysis (Baron et al., 2017), frequency of

rs6313 homozygous variant carriers was highest in patients with mechanical hyperalgesia (cluster MH), while frequency of homozygous wild-type carriers was highest in patients with thermal hyperalgesia (cluster TH). This underpins our results of an increased sensitivity to mechanical pain for the variant allele.

Finally, rs6313 explains a similar amount of variance for both evoked (pinprick) pain and ongoing spontaneous pain. The expression of gain in the PC2 somatosensory component interpreted as being related to the occurrence of CS was significantly associated with more pain using various analyses. Thus, this is the first study showing that a change of somatosensory profile associated with a SNV can be tentatively interpreted as evidence that CS can lead to higher pain owing to dysfunction of descending pain control.

As a limitation, there is no validation group provided in our study. Our results should be verified in a larger patient cohort, including also other genes that may interact with *HTR2A* in influencing the somatosensory phenotype of neuropathic pain. However, patients were well-phenotyped using standardized QST. Although an impact of patients' medication on QST results cannot be fully excluded, a recently published study in 1135 peripheral neuropathic pain patients suggests no significant impact of drugs on sensory profiles (Baron et al., 2017). Since phenotypical characterization of healthy controls was not performed in this study, the influence *HTR2A* variants on the somatosensory function in general could not be determined. Due to the small sample size, subgroup analyses of underlying disease entities were not performed. However, the individual somatosensory profile is thought to reflect the underlying pain-generating mechanisms better than disease entities. Improvement of neuropathic pain therapy includes the development of drugs selectively targeting these mechanisms (Baron et al., 2017; Binder & Baron, 2015; Binder et al., 2011). The present study focused on the concept of mechanism-based therapy by analysing different subgroups of patients.

In summary, our study suggests that the genetic variant *HTR2A* rs6313 and the linked variant rs6311 increase the progress of pinprick hyperalgesia as surrogate of CS in neuropathic pain patients. These effects seem to depend on the somatosensory phenotype and is mainly seen in loss of peripheral afferent small and large fibre function. Thus, our results present a promising avenue for future drug development and might help to realize the concept of mechanism-based therapy.

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CONFLICT OF INTEREST

JS reports personal fees from Grünenthal GmbH and travel support from Alnylam Pharmaceuticals and Pfizer outside the submitted work. JG has received speaker fees and travel support from Pfizer, Novartis, Grünenthal GmbH, Sanofi Pasteuer MSD GmbH, TAD Pharma and consultancy fees from Glenmark, outside this work. WM received grants from the DFG and BMBF during the conduct of the study outside the submitted work. In addition, WM has a patent DE 103 31 250.1–35 on pinprick stimulators with royalties paid by MRC Systems. AB received honoraria from Grünenthal, Allergan and Pfizer, outside this work. JF reports personal fees and non-financial support from Grünenthal GmbH and Sanofi Genzyme, personal fees from Bayer and Imedu, non-financial support from Novartis, outside the submitted work. RDT received grants from European Union and EFPIA companies, Pfizer and BMBF during the conduct of the study; grants from Boehringer Ingelheim, Astellas, AbbVie, Bayer and Teva as well as personal fees from Astellas, Grünenthal, Bauerfeind, Hydra and Bayer outside the submitted work. In addition, RDT has a patent DE 103 31 250.1–35 on pinprick stimulators with royalties paid by MRC Systems. Abe (Berthele) received honoraria from Pfizer, outside this work. SR reports speaker fees from Bayer Vital GmbH, outside this work. SCF reports personal fees from Grünenthal GmbH, outside this work. JV has received consultancy fees from Casquar, outside this work. RR received research grants by BMBF (01GY1703), by the Innovation Fund of the Joint Federal Committee (01VSF16007) and Robert Bosch Foundation (01000039-008). He received speaker or consultant fees from AOP Orphan, Astellas Pharma, Bionorica ethics, Grünenthal GmbH, Lilly, Pfizer, Tilray. CS has received honoraria related to educational talks or consulting in the area of pain from Air Liquide, Algiax, Bayer, Grünenthal and Pfizer, outside this work. IC received honoraria from Astellas, Daichi-Sankyo and UCB-Pharma, outside this work. RB reports grants/research support from Pfizer, Genzyme GmbH, Grünenthal GmbH, from the DOLORisk project (633491), from the IMI 'Europain' collaboration, the German Federal Ministry of Education and Research (BMBF; DFNS, 01EM0903), the NoPain system biology project (0316177C) and the ERA_NET NEURON/IM-PAIN Project (01EW1503). He has received speaker fees from Pfizer, Genzyme GmbH, Grünenthal GmbH, Mundipharma, Sanofi Pasteur, Medtronic Inc. Neuromodulation, Eisai Co. Ltd., Lilly GmbH, Boehringer Ingelheim Pharma GmbH&Co. KG, Astellas, Desitin, Teva Pharma, Bayer-Schering, MSD GmbH, Seqirus, Novartis, TAD Pharma GmbH; he has been a consultant for Pfizer, Genzyme GmbH, Grünenthal GmbH, Mundipharma, Allergan, Sanofi Pasteur, Medtronic, Eisai, Lilly GmbH, Boehringer Ingelheim Pharma GmbH&Co. KG, Astellas, Novartis, Bristol-Myers Squibb, Biogenidec, AstraZeneca, Merck, Abbvie, Daiichi Sankyo, Glenmark

Pharmaceuticals, Seqirus, Teva Pharma, Genentech, Galapagos NV, Kyowa Kirin GmbH, Vertex Pharmaceuticals Inc., Biotest AG, Celgene, Desitin, Theranexus, Abbott, Bayer AG, Akcea, Asahi Kasei Pharma, AbbVie GmbH, Air Liquide, Alnylam GmbH, Lateral Pharma Pty Ltd, Hexal AG. HB, DM, JK, TRT, CM (Maier), AC, CD, HF, VH, WM, CMaih (Maihöfner), DK, SS and AW declared no conflicts of interest.

AUTHORS' CONTRIBUTIONS

JS has performed the data analysis, data interpretation and manuscript preparation. HB and JG have contributed to the data interpretation and manuscript preparation. WM contributed to the data analysis, interpretation and manuscript preparation. DM has made the experimental design. AB has contributed to the study concept and design, phenotype data acquisition, data interpretation and manuscript preparation. JF has contributed to the data analysis. JK has contributed to genotyping of all samples. RDT contributed to data interpretation and manuscript preparation. ABe contributed to the acquisition and preparation of DNAs, reviewed the manuscript for intellectual content and approved the final version. AC has supported statistical analysis. CD has contributed to genotyping of all samples. JV has performed the statistical analysis of the three clusters. RR has contributed to the data interpretation and manuscript preparation. AW collected the patients and data for the DFNS data base, read and approved the manuscript. IC has contributed to the study concept and design, data interpretation and manuscript preparation. RB has contributed to the study concept and design, data interpretation and manuscript preparation. CM (Maier), TRT, HF, VH, CMaih (Maihöfner), SR, DK, SF, SS and CS reviewed and approved the manuscript. All authors have access to all data in this study and hold final responsibility for the decision to submit this manuscript for publication.

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