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WORD COUNT	4491	TIME SUBMITTED	16-APR-2022 01:28PM
		PAPER ID	84870217

8
Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 69393

Manuscript Type: ORIGINAL ARTICLE

Retrospective Study

Serotonin Type 3 Receptor Subunit Gene Polymorphisms Associated with Psychosomatic Symptoms in Irritable Bowel Syndrome: a Multicenter Retrospective Study

5-HT3 Receptor Gene Polymorphisms in IBS

Abstract

BACKGROUND

Single-nucleotide polymorphisms (SNPs) of the serotonin type 3 receptor subunit (*HTR3*) genes have been associated with psychosomatic symptoms, but it is not clear whether these associations exist in irritable bowel syndrome (IBS).

AIM

In this study, we assessed the association of *HTR3* polymorphisms with depressive, anxiety, and somatization symptoms in individuals with IBS.

METHODS

In this retrospective study, 623 participants with IBS were recruited from five specialty centers in Germany, Sweden, the US, the UK, and Ireland. Depressive, anxiety, and somatization symptoms and sociodemographic characteristics were collected. Four functional SNPs – *HTR3A* c.-42C>T, *HTR3B* c.386A>C, *HTR3C* c.489C>A, and *HTR3E* c.*76G>A – were genotyped and analyzed using the dominant and recessive models. We also performed separate analyses for sex and IBS subtypes. SNP scores were

calculated as the number of minor alleles of the SNPs above. The impact of *HTR3C* c.489C>A was tested by radioligand-binding and calcium influx assays.

RESULTS

Depressive and anxiety symptoms significantly worsened with increasing numbers of minor *HTR3C* c.489C>A alleles in the dominant model ($F_{depressive}$ = 7.475, $p_{depressive}$ = 0.006; $F_{anxiety}$ = 6.535, $p_{anxiety}$ = 0.011). A higher SNP score (range 0–6) was linked to a worsened depressive symptoms score (F = 7.710, *p-linear-trend* = 0.006) in IBS. The potential relevance of the *HTR3C* SNP was corroborated, showing changes in the expression level of 5-HT_3AC variant receptors.

CONCLUSION

We have provided the first evidence that *HTR3C* c.489C>A is involved in depressive and anxiety symptoms in individuals with IBS. The SNP score indicated that an increasing number of minor alleles is linked to the worsening of depressive symptoms in IBS.

Key Words: Irritable Bowel Syndrome; 5-HT3 Receptor Subunit Gene Polymorphisms; SNP Score; Depression; Anxiety; Somatization

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Core Tip: Bringing together high quality data as well as methodological expertise, our results show that (1) in the dominant model, HTR3C c.489C>A was correlated with depressive and anxiety symptoms in IBS; (2) a higher number of minor alleles (i.e., the higher the SNP score, which was computed by combining the individual SNP status of HTR3A c.-42C>T, HTR3B c.386A>C, HTR3C c.489C>A, and HTR3E c.*76G>A) was linked to more severe depressive symptoms in IBS; and (3) the potential relevance of the HTR3C SNP was corroborated in functional assays showing changes in the expression level of 5-HT3AC variant receptors. We are confident that these results are of interest to your readership, as they contribute substantially to update current knowledge regarding the role of accumulation of HTR3 SNPs in depressive and anxiety symptoms in IBS patients. In turn, our data will contribute towards standardization and harmonization of genetic research strategies in IBS.

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal (GI) disorder characterized by abdominal pain and altered bowel habits[1-4]. The pathophysiology of IBS has not entirely been resolved, but is understood to be biopsychosocial and affected by an impaired function of the central and enteric nervous systems and their crosstalk *via* the brain-gut axis[5,6]. IBS patients often present with increased comorbid depressive and anxiety symptoms[7-11], highlighting the complex relationship between visceral sensitivity and subjective psychological perceptions[12,13]. Nevertheless, about 50% of IBS patients report GI symptoms but show no comorbid affective symptoms[14].

There is evidence that disturbances of the serotonergic system are important in GI disorders such as IBS and in mental disorders, both of which interact *via* the brain-gut axis[15,16]. The serotonin type 3 receptors (5-HT_3R) modulate key functions in the GI tract[17,18]. In line with such functions, 5-HT_3R antagonists are beneficial in the treatment of diarrhea-predominant IBS (IBS-D)[19-22]. 5-HT_3Rs are also involved in emotional processing, mood regulation, and visceral perception and have been associated with depressive and anxiety symptoms that represent comorbid phenotypes in IBS[23]. Single-

nucleotide polymorphisms (SNPs) in the serotonin type 3 receptor subunit genes (*HTR3*), namely *HTR3A* c.-42C>T (rs1062613), *HTR3B* c.386A>C (rs1176744), *HTR3C* c.489C>A (rs6766410), and *HTR3E* c.*76G>A (rs56109847), are associated with IBS according to studies investigating the effects of sex or IBS subtypes[12,24-30]. However, whether *HTR3* polymorphisms are associated with IBS and comorbid depressive and anxiety symptoms has not been determined because existing studies have missing phenotypic data on comorbidities and small sample sizes. These studies had case-control designs and investigated associations between these polymorphisms in individuals with IBS phenotypes or mental behavioral conditions and controls rather than combining genetic data with specific psychosocial characteristics of IBS patients.

This multicenter observational study focused on a large IBS patient cohort comprising 768 participants from centers in Germany, Sweden, the US, the UK, and Ireland with the aim of meeting three objectives: (1) to explore the associations between functional *HTR3* polymorphisms and psychosomatic burden (i.e., depressive, anxiety, and somatization symptoms) within an IBS population; (2) to investigate the impact of the *HTR3* SNP score (computed as the number of minor alleles) on psychosomatic burden, based on our hypothesis that the observed number of minor alleles was associated with specific mental characteristics in IBS patients; and (3) to perform a functional analysis of variant 5-HT_3AC receptors.

MATERIALS AND METHODS

2.1 Subjects

The study population was pooled from five different tertiary care expert centers. German participants were recruited from the Specialty Clinic for Functional GI Disorders at the Department of General Internal Medicine and Psychosomatics of Heidelberg University Hospital[31] and from our clinical partners in the IBS-Net in Hamburg, Krefeld, Berlin, Vilsbiburg, and Munich (www.ibs.uni-hd.de). Swedish participants were recruited at the specialized unit for patients with functional GI disorders at Sahlgrenska University Hospital in Gothenburg. US participants were

recruited at Washington University, Barnes-Jewish Hospital in St. Louis, Missouri. UK participants were recruited at the Nottingham Digestive Diseases Center and participants from Ireland from a specialty clinic at Cork University Hospital. Participant recruitment is shown in Figure 1.

Written informed consent was obtained from all participants and the experiments were in accordance with the principles of the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. All studies were approved by the following local Ethics Committees: Heidelberg, Germany: Ethical Committee, Medical Faculty of the Heidelberg University Hospital (S067/2010); Cork, Ireland: Clinical Research Ethics Committee (APC024); Gothenburg, Sweden: Regional Ethical Review Board in Gothenburg (S489-02 and 731-09); Nottingham, UK: registered at clinical trial clinicaltrials.gov (identifier NCT00745004) and approved by Nottingham Research Ethics Committee 2 (REC reference number 08/H0408/134)[21]; and St-Louis, US: Washington University St. Louis, Human Research Protection Office (IRB ID #: 201103220).

Inclusion/exclusion criteria

Only patients diagnosed with IBS according to the ROME III criteria were included in the analysis. All participants were of Caucasian ancestry and had comparable population stratification. Patients under 18 years of age or without SNP test results were excluded.

2.2 Measures

2.2.1 Genotyping

Genomic DNA was isolated from IBS patient blood samples using ethylenediaminetetraacetic acid (EDTA) according to standard protocols[32]. Four polymorphic *HTR3* loci, namely *HTR3A* c.-42C>T (rs1062613), *HTR3B* c.386A>C (rs1176744), *HTR3C* c.489C>A (rs6766410), and *HTR3E* c.*76G>A (rs56109847) were selected as target SNPs for this study. The corresponding primers were designed and synthesized using AssayDesigner 3.1 software. Genotyping was performed at the Department of Human Molecular Genetics at Heidelberg University Hospital using the

KASPar® SNP Genotyping System (KBiosciences, Ltd, Hoddesdon, UK). To analyze *HTR*3 SNPs, the fluorescence plate reader of the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, California, US) was used as recommended. About 10% of the samples were repeat tested to ensure genotyping accuracy.

2.2.2 Data collection

In addition to sociodemographic characteristics and IBS diagnosis, we also collected data on depressive, anxiety, and somatization symptoms[33] and genetic markers of the serotonergic system.

IBS diagnosis: The diagnostic classification of IBS was based on the Scoring Algorithm for Rome III Diagnostic Questionnaire for Adult Functional GI Disorders (SA for Rome III-DQ)[34,35] in all five centers. Percentages of the different IBS subtypes, i.e., constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), IBS with mixed bowel habits (IBS-M), and unclassified IBS (IBS-U), were also calculated.

Depressive symptoms: The nine-item depression module from the Patient Health Questionnaire (PHQ-9)[36,37] was used to measure depressive symptoms in the German cohort. The Hospital Anxiety and Depression Scale depression subscale (HADS-D)[38] was used to identify depressive symptoms in participants from Sweden, the UK, and Ireland and the Beck Depression Inventory (BDI)[39] was used to measure the severity of depressive symptoms in US participants.

Anxiety symptoms: In the German cohort, symptoms of generalized anxiety were assessed using the brief measurement for generalized anxiety disorder (GAD-7)[40]. In the cohorts from Sweden, the UK, and Ireland, the HADS anxiety subscale (HADS-A) was used to identify anxiety symptoms. The Beck Anxiety Inventory (BAI)[41] was used to assess anxiety in the US cohort.

Somatization symptoms: In the cohorts from Germany, Sweden, and the US, the 15-item somatization module from the PHQ-15[42] was used to identify somatization symptoms.

Genetic markers of the serotonergic system: The four functional SNPs *HTR3A* c.-42C>T (rs1062613), *HTR3B* c.386A>C (rs1176744), *HTR3C* c.489C>A (rs6766410), and *HTR3E*

c.*76G>A (rs56109847) were selected for validation based on previous reports as outlined above[25].

2.2.3 Ligand binding and calcium influx assays

These procedures are described in the supplementary data.

2.3 Statistical analyses

All statistical procedures were carried out using IBM SPSS Statistics 22.0 for Windows. Variables with a skewed distribution were log-transformed prior to further analysis. If different measurements had been collected in the five centers, *z* values were calculated to enable pooling. The Hardy–Weinberg equilibrium (HWE) of genotype frequency distribution was tested using SHEsis[43]. Genome-wide SNP data were generated by the Bellygenes team of Mauro D'Amato using the Illumina Global Screening array and platform[44,45]. We used the multidimensional scaling approach to correct for population stratification in PLINK[46]. Following the guidance provided at *https://github.com/MareesAT/GWA_tutorial/*, our data were anchored by data of the 1000 Genomes project (http://www.1000genomes.org/). The 10 main components were used as covariates in the association tests to correct for population stratification[46] and exclude outliers. Polymorphisms were analyzed separately using the dominant and the recessive models. Also, stratified analyses based on sex and IBS subtypes were carried out. ANOVA was used to analyze group differences and to check for linear trends in depressive, anxiety, and somatization symptoms. For the independent variable, a SNP score was computed based on the number of minor alleles (i.e., continuous from 0 to 8 for the four SNPs). Based on the first human *HTR3* locus-specific variant database (www.htr3.uni-hd.de) [25], scoring criteria were as follows: major allele homozygous variant gene = 0; heterozygous variant gene = 1; and minor allele homozygous variant gene = 2 (for details see Table 1). Statistical comparisons were made between the two groups using the χ^2 test or Fisher's exact test for frequencies and *t* tests or Mann-Whitney *U* tests for metric variables. Normal distribution and variance homogeneity were checked as conditions. Statistical tests were two-sided based on an alpha error of 0.05%. All analyses were explorative and not confirmatory. False discovery rates (FDRs)

were calculated based on overall p values using the Benjamini–Hochberg method[47]. Significant values that were no longer significant after FDR multiple testing correction were named "nominally significant".

RESULTS

3.1 Sociodemographic and symptomatic characteristics

In total, 623 participants from five independent expert centers were included in this study (45.1% from Germany, 18.3% from Sweden, 19.6% from the US, 12.4% from the UK, and 4.7% from Ireland). We excluded 76 Swedish participants who did not meet the population stratification criteria (Supplementary Figure 1). Participants from the UK and Ireland were excluded from the main analysis because the sample size was small. The analyses of these participants are presented separately in the supplementary data. Table 2 presents the sociodemographic characteristics, IBS subtypes, and psychosomatic symptoms of the included participants. Participants had a mean±standard deviation age of 41.7±16.1 years and 69.5% were female. Overall, IBS patients showed minimal to mild levels of depressive and anxiety symptoms, moderate levels of IBS symptoms, and moderate levels of somatization symptoms.

3.2 *HTR3* SNP genotypes and allele frequencies

Genotype and allele frequencies of the functional *HTR3A* c.-42C>T, *HTR3B* c.386A>, *HTR3C* c.489C>A, and *HTR3E* c.*76G>A polymorphisms were calculated. No significant differences in genotype frequency were observed between sexes or IBS subtypes. For *HTR3A* c.-42C>T, the frequency of the minor *T* allele was 21.5%; for *HTR3B* c.386A>C, the frequency of the minor *C* allele was 29.6%; for *HTR3C* c.489C>A, the frequency of the minor *A* allele was 41.6%; and for *HTR3E* c.*76G>A, the frequency of minor *A* allele was 6.1%. The genotypic distribution of the four polymorphic loci of rs1062613, rs1176744, rs6766410, and rs56109847 were in accordance with HWE (all $p > 0.05$). The results are shown in Supplementary Tables 1, 2, and 3.

3.3 *HTR3* SNP analysis using the dominant and the recessive model

HTR3 SNPs were separately analyzed using the dominant model and the recessive model stratified for sex and IBS subtypes. Depressive and anxiety symptoms worsened significantly with increasing numbers of minor alleles of *HTR3C* c.489C>A in the dominant model ($F_{depressive}$ = 7.475, $p_{depressive}$ = 0.006; $F_{anxiety}$ = 6.535, $p_{anxiety}$ = 0.011). This seemed to be driven by female sex ($F_{depressive}$ = 7.040, $p_{depressive}$ = 0.008; $F_{anxiety}$ = 7.550, $p_{anxiety}$ = 0.006) and IBS-D ($F_{depressive}$ = 5.670, $p_{depressive}$ = 0.018; $F_{anxiety}$ = 13.444, $p_{anxiety}$ < 0.001). The same trend was also found for *HTR3A* c.-42C>T in male participants with depressive symptoms in the dominant model ($F_{depressive}$ = 4.149, $p_{depressive}$ = 0.043). For the recessive model, depressive and somatization symptoms worsened with increasing numbers of minor alleles of *HTR3C c.489C>A* ($F_{depressive}$ = 6.190, $p_{depressive}$ = 0.014) and *HTR3B c.386A>C* ($F_{depressive}$ = 6.482, $p_{depressive}$ = 0.011), respectively in IBS-D participants. *F* values from the ANOVA are shown in Table 3.

3.3 Effect of SNP score on psychosomatic symptoms

SNP scores ranged from 0 to 6; 37.1% had one or zero minor alleles of the analyzed *HTR3* SNPs and 30.8% had three or more minor alleles of the analyzed *HTR3* SNPs. No significant differences in SNP scores were observed between sexes (F = 3.550, p = 0.060) or IBS subtypes (F = 1.485, p = 0.227). ANOVAs were conducted and linear trends were checked to analyze the effect of SNP scores on depressive, anxiety, and somatization symptoms. Overall, an increasing number of minor alleles was linked to worsening depressive symptoms (F = 7.710, *p-linear trend* = 0.006). However, material trends did not reveal a link between more minor alleles and worsening anxiety or somatization symptoms. By stratifying analyses for sex, an increasing number of minor alleles was linked to worsened depressive symptoms in female participants (F = 5.770, *p-linear trend* = 0.017). There was no significant association between SNP score and depressive, anxiety, or somatization symptoms when looking at IBS subtypes separately (Table 4).

3.4 Functional analysis of variant 5-HT_3AC receptors

The *HTR3C* SNP encodes the amino acid exchange p.Asn163Lys (p.N163K), and recombinantly expressed 5-HT_3AC receptors harboring variant 5-HT3C subunits that

mimic the homozygous minor allele and the heterozygous state presented with increased cell surface expression and enhanced 5-HT maximum response. Radioligand-binding assays revealed higher B_{max} values of 117.1±4.38% and 111.9±1.79%. Calcium influx assays showed increased 5-HT-induced maximum effects of 137.2±9.0% and 151.9±17.3% for the minor allele 5-HT_3AC 163K or combined 5-HT_3AC 163N/5-HT_3AC 163K receptors compared with the major allele representing 5-HT_3AC 163N receptors, respectively. The affinity of the specific 5-HT_3 receptor antagonist [^{3}H]GR65630, as reflected by the Kd values, and the potency of 5-HT, as reflected by the EC_{50} values, did not differ between the receptor variants (Figure 2).

DISCUSSION

4.1 Main findings

The 5-HT_3 receptors modulate essential functions in the GI tract such as GI motility as well as mood and emotions[19], and *HTR3* SNPs have been associated with depression, anxiety, and IBS[25]. In this study, we showed that: (1) in the dominant model, *HTR3C c.489C>A* was correlated with depressive and anxiety symptoms in IBS; (2) a higher number of minor alleles (i.e., a higher SNP score, which was computed by combining the individual SNP status of *HTR3A c.-42C>T, HTR3B c.386A>C, HTR3C c.489C>A*, and *HTR3E c.*76G>A*) was linked to more severe depressive symptoms in IBS; and (3) the potential relevance of the *HTR3C* SNP was corroborated in functional assays showing changes in the expression level of 5-HT_3AC variant receptors. These findings are discussed in more detail below.

4.1.1 Sample characteristics

Participants with IBS were more frequently female than male, in line with previous findings[48,49] that IBS is more prevalent in young and middle-aged females. Most participants with IBS were only mildly affected by depression and anxiety symptoms. Of note, German participants only visited the Specialty Clinic for Functional GI Disorders at Heidelberg University Hospital after a long history of dealing with IBS[31]; therefore, these participants reported more severe depressive symptoms. However,

causal relationships between IBS symptoms, depression, and anxiety are still controversial.

The investigated SNPs rs1062613, rs1176744, rs6766410, and rs56109847 were in accordance with HWE. There was no population stratification, and the sample was representative and excluded genotyping errors. Although participants were recruited from various centers in different countries, there were no obvious selective differences.

4.1.2 Connections between *HTR3* SNPs and mental symptoms

***HTR3A* c.-42C>T (rs1062613)**: Depressive symptoms were "nominally significantly" more severe with increasing numbers of minor *HTR3A* c.-42C>T alleles in male participants according to the dominant model. This SNP has been associated with bipolar affective disorder[50], IBS symptom severity, amygdaloid activity[29,51], early life trauma[52], altered emotional networks in the human brain, and the onset of depression[53]. However, these studies only included single sex participants and did not include subgroup analyses.

***HTR3B* c.386A>C (rs1176744)**: Somatization symptoms worsened significantly with increasing numbers of minor *HTR3B c.386A>C* alleles in the dominant model. The *HTR3B* variant p.Tyr129Ser (rs1176744) has been associated with bipolar affective disorder in males and with major depression in females as well as with pain catastrophizing, a coping style characterized by excessively negative thoughts and emotions related to pain[30,54-56]. This discrepancy may be due to an enhancement or weakening of this association by polymorphic interactions in the serotonin pathways[56].

***HTR3C* c.489C>A (rs6766410)**: Depressive and anxiety symptoms worsened significantly with increasing numbers of minor *HTR3C* c.489C>A alleles in the dominant model. This effect seemed to be driven by female sex and IBS-D. *HTR3C* c.489C>A was previously associated with IBS-D in female patients[57], but the proportion of male patients was small in this study, which may limit the applicability of these findings. In the recessive model, depressive and anxiety symptoms "nominally significantly" worsened with increasing numbers of minor alleles of *HTR3C* c.489C>A

in Irish participants. However, these results should be interpreted with caution because the Irish sample size was low. As far as we are aware, *HTR3C* c.489C>A has not been analyzed in individuals with affective disorders before.

HTR3E* c.*76G>A (rs56109847): *HTR3E* is restrictedly and robustly expressed in the GI tract[58,59], suggesting that it plays a special role in 5-HT_3 receptor function in the gut. In this study, we did not find a relationship between functional polymorphisms of *HTR3E* and depressive and anxiety symptoms in IBS patients. This may be attributed to a floor effect because depressive and anxiety symptoms were minimal to mild in our sample[60].

4.1.3 The SNP score and its impact on depressive symptoms

A single gene variant is not sufficient to explain all symptoms shaping the clinical phenotype of a complex disorder like IBS[61]. By computing SNP scores based on the number of minor alleles of rs1062613, rs1176744, rs6766410, and rs56109847, our study revealed that an increasing number of minor alleles is linked to increasing severity of depressive symptoms. However, there was no obvious association between an increasing number of minor alleles and the severity of anxiety or somatization symptoms. Stratification for sex revealed a correlation between increasing numbers of minor alleles and worsening depressive symptoms in female participants.

4.1.4 Functional properties of variant 5-HT_3AC receptors

HTR3 genes encode different 5-HT3 subunits to make up heteromeric receptors. The 5-HT3A subunits play a major role in these receptors because they can form functional receptors on their own. The other subunits can only form functional receptors with 5-HT3A and seem to modulate the function and properties of the receptors[62]. How these native receptors might contribute to the pathogenesis of IBS, particularly regarding co-expression patterns of *HTR3*, has not been established yet. The *HTR3A* and *HTR3E* variants reside within untranslated regions and the respective SNPs correlate with increased expression levels, whereas the *HTR3B* variant changes the channel properties[25]. To gain insight into the pathophysiological relevance of the associated *HTR3C* variant c.489C>A (rs6766410), we characterized the pharmacological and functional properties of those 5-HT_3AC receptors that altered the 5-HT-mediated

maximum response and expression of variant 5-HT_3AC receptors. However, how structural modifications in these receptors affect their function *in vivo* and how they modulate the serotonergic system to influence mood, emotional processing, and the manifestation of IBS and comorbid conditions remains to be determined.

4.2 Limitations and strengths

Our study has some limitations. First, different instruments were used by different centers to assess phenotypic features. To correct for this, scale scores were converted into *z* standard scores. However, given that the participants reported no severe psychosomatic symptoms, the discovery chances might be limited. Second, there is no sufficient evidence to show the relationship between risk alleles and respective major/minor alleles as patients and healthy controls were not compared in this study. Similarly, the relative strength of the cumulative effect represented by the SNP score was also affected to a certain extent. Third, our participants were all Caucasian, so the results may not apply to other ethnic groups.

Despite these limitations, our study has some strengths. First, this was a multicenter study so had a large sample size. Large, well-characterized samples like ours are necessary to identify molecular causes of IBS and comorbid conditions [12]. Second, this study investigated the association between polymorphisms in *HTR3* genes and comorbid psychosomatic symptoms for the first time. We conducted population stratification tests to ensure that the included populations were comparable. We also performed stratified analyses of sex and IBS subtypes and a more stringent multiple testing correction by FDR. Third, SNP scores have higher power and are better suited to testing multiple instead of single variants. This is useful because the pathogenesis of IBS is complex with multiple factors contributing to the manifestation of various subtypes. Also, individual genes may only play a minor role [12].

4.3 Clinical implications and further research

IBS is a complex condition. The continuous improvement of the allelic variation database for *HTR3* [25] and deep phenotyping combined with gene information (also in

other datasets) may help to identify disease subgroups accurately and consistently, thereby facilitating future treatment[33,63]. This will be an important step towards standardization and unification of IBS genetic research strategies.

CONCLUSION

4.4 Conclusions

Our results provide the first evidence that the accumulation of *HTR3* SNPs (reflected by the SNP score computed by *HTR3A* c.-42C>T, *HTR3B* c.386A>C, *HTR3C* c.489C>A, and *HTR3E* c.*76G>A) may play a role in the pathophysiology of depressive and anxiety symptoms in IBS. This study has revealed that 1) depressive and anxiety symptoms significantly worsened from the major to the minor allele of *HTR3C* c.489C>A in the dominant model and 2) an increasing number of minor alleles are linked to more severe depressive symptoms in IBS.

ARTICLE HIGHLIGHTS

Research background

Over the past decades, genetic evidence on the key players within the serotonergic system including the serotonin type 3 (5-HT3) receptor subunit genes (*HTR3*) accumulated showing association with irritable bowel syndrome (IBS) as well as mental illnesses. However, it has never been explored whether associations of the single-nucleotide polymorphisms (SNPs) of *HTR3* genes to depressive and anxiety symptoms can be replicated within IBS.

Research motivation

In order to address this knowledge gap, This multicenter observational study focused on a large IBS patient cohort comprising 768 participants from centers in Germany, Sweden, the US, the UK, and Ireland.

Research objectives

(1) to explore the associations between functional *HTR3* polymorphisms and psychosomatic burden within an IBS population; (2) to investigate the impact of the *HTR3* SNP score on psychosomatic burden, based on our hypothesis that the observed number of minor alleles was associated with specific mental characteristics in IBS patients; and (3) to perform a functional analysis of variant 5-HT_3AC receptors.

Research methods

In this retrospective study, 623 participants with IBS were recruited from five specialty centers in Germany, Sweden, the US, the UK, and Ireland. Depressive, anxiety, and somatization symptoms and sociodemographic characteristics were collected. Four functional SNPs – *HTR3A* c.-42C>T, *HTR3B* c.386A>C, *HTR3C* c.489C>A, and *HTR3E* c.*76G>A – were genotyped and analyzed using the dominant and recessive models. We also performed separate analyses for sex and IBS subtypes. SNP scores were calculated as the number of minor alleles of the SNPs above. The impact of *HTR3C* c.489C>A was tested by radioligand-binding and calcium influx assays.

Research results

Bringing together high quality data as well as methodological expertise, our results show that (1) in the dominant model, *HTR3C c.489C>A* was correlated with depressive and anxiety symptoms in IBS; (2) a higher number of minor alleles (i.e., the higher the SNP score, which was computed by combining the individual SNP status of *HTR3A c.-42C>T, HTR3B c.386A>C, HTR3C c.489C>A*, and *HTR3E c.*76G>A*) was linked to more severe depressive symptoms in IBS; and (3) the potential relevance of the *HTR3C* SNP was corroborated in functional assays showing changes in the expression level of 5-HT_3AC variant receptors.

Research conclusions

Our results provide the first evidence that the accumulation of *HTR3* SNPs (reflected by the SNP score computed by *HTR3A* c.-42C>T, *HTR3B* c.386A>C, *HTR3C* c.489C>A, and

HTR3E c.*76G>A) may play a role in the pathophysiology of depressive and anxiety symptoms in IBS.

Research perspectives

We are confident that these results are of interest to your readership, as they contribute substantially to update current knowledge regarding the role of accumulation of *HTR3* SNPs in depressive and anxiety symptoms in IBS patients. In turn, our data will contribute towards standardization and harmonization of genetic research strategies in IBS.

ACKNOWLEDGEMENTS

We would like to thank all patients for their participation in this study and the supporting staff at each site. We acknowledge the kind support of Claus R. Bartram and Katrin Hinderhofer. We would also thank Beatrix Startt for proofreading and Claire Bacon for editing the manuscript. This manuscript results in part from collaboration and network activities promoted under the frame of the international network GENIEUR (*Genes in Irritable Bowel Syndrome Research Network Europe*), which has been funded by the COST program (BM1106, www.GENIEUR.eu) and is currently supported by the *European Society of Neurogastroenterology and Motility* (ESNM, www.ESNM.eu).

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