Serotonin receptor 3A polymorphism c.-42C > T is associated with severe dyspepsia

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Abstract

Background: The association between anxiety and depression related traits and dyspepsia may reflect a common genetic predisposition. Furthermore, genetic factors may contribute to the risk of having increased visceral sensitivity, which has been implicated in dyspeptic symptom generation. Serotonin (5-HT) modulates visceral sensitivity by its action on 5-HT3 receptors. Interestingly, a functional polymorphism in HTR3A, encoding the 5-HT3 receptor A subunit, has been reported to be associated with depression and anxiety related traits. A functional polymorphism in the serotonin transporter (5-HTT), which terminates serotonergic signalling, was also found associated with these psychometric comorbidities and increased visceral sensitivity in irritable bowel syndrome, which coexistence is associated with higher dyspeptic symptom severity. We investigated the association between these functional polymorphisms and dyspeptic symptom severity.

Methods: Data from 592 unrelated, Caucasian, primary care patients with dyspepsia participating in a randomised clinical trial comparing step-up and step-down antacid drug treatment (The DIAMOND trial) were analysed. Patients were genotyped for HTR3A c.-42C > T SNP and the 44 bp insertion/deletion polymorphism in the 5-HTT promoter (5-HTTLPR). Intensity of 8 dyspeptic symptoms at baseline was assessed using a validated questionnaire (0 = none; 6 = very severe). Sum score ≥20 was defined severe dyspepsia.

Results: HTR3A c.-42T allele carriers were more prevalent in patients with severe dyspepsia (OR 1.50, 95% CI 1.06-2.20). This association appeared to be stronger in females (OR 2.05, 95% CI 1.25-3.39) and patients homozygous for the long (L) variant of the 5-HTTLPR genotype (OR 2.00, 95% CI 1.01-3.94). Females with 5-HTTLPR LL genotype showed the strongest association (OR = 3.50, 95% CI = 1.37-8.90).

Conclusions: The HTR3A c.-42T allele is associated with severe dyspeptic symptoms. The stronger association among patients carrying the 5-HTTLPR L allele suggests an additive effect of the two polymorphisms. These results support the hypothesis that diminished 5-HT3 mediated antinociception predisposes to increased visceral sensitivity of the gastrointestinal tract. Moreover, the HTR3A c.-42C > T and 5-HTTLPR polymorphisms likely represent predisposing genetic variants in common to psychiatric morbidity and dyspepsia.
Furthermore, psychosocial factors and psychiatric morbidity are underlying risk factors for the development of dyspeptic symptoms [13]. The most common psychiatric comorbidities in patients with dyspepsia are anxiety and depressive disorders [14].

Several genetic variants have been reported to affect the risk of having dyspepsia [15-19]. The mechanism underlying the association with the C825T polymorphism in the gene encoding the G protein β3 subunit remains to be determined [15-17]. Abnormal immune response against H. pylori is likely underlying the associations with RANTES promoter C-28G genotype and Toll-like receptor 2 -196 to -174 del carrier status [18,19]. There is evidence of genetic influence on other risk factors for dyspepsia, i.e. psychosocial factors and psychiatric morbidity [20]. The association between psychosocial factors, psychiatric morbidity and dyspepsia may reflect a common genetic predisposition. Furthermore, we hypothesized that genetic factors may contribute to the risk of having increased visceral sensitivity and (consequently) affect the intensity of dyspepsia.

Serotonin (5-HT) plays a key role in modulating upper gastrointestinal sensory function [21]. Besides, central alterations in 5-HT transmission are thought to have a role in anxiety and depression [22]. Therefore, genes of the serotonergic system are critical candidates in assessing the role of genetic factors in dyspeptic symptom severity. Of special interest is the 5-HT3 receptor, as 5-HT3 receptor antagonist reduces dyspeptic symptoms [23,24] and exerts anxiolytic effects [25]. The 5-HT3 receptor is a ligand-gated ion channel, structured as a pentameric complex. In humans, five different subunit genes, HTR3A-E, have been identified [26]. The 5-HT3A subunit seems to play a key role in receptor formation, since it is the only subunit that can form functional homopentamers. The other subunits only form functional heteromers with the 5-HT3A subunit [26]. A functional polymorphism, c.-42C > T (rs1062613), has been identified in the HTR3A gene. The T allele promotes translation of the transcript [27,28]. It is noteworthy that the c.-42C > T polymorphism has been reported associated with depressive disorder [27], the anxiety-related trait harm avoidance [29], and irritable bowel syndrome (IBS), a functional gastrointestinal disorder showing comorbidity with anxiety and depression and patients displaying visceral hypersensitivity [28,30].

Serotonergic signalling is terminated, peripherally and centrally, by 5-HT transporter (5-HTT) mediated uptake. A common polymorphism, a 44 base pair (bp) insertion/deletion, has been described in the promoter (transcriptional control region) of the 5-HTT gene. This polymorphism, 5-HTTLPR, creates a long (L) and a short (S) allele [31]. Homozygosity for the short variant and heterozygosity result in reduced transcription, less protein expression and less reuptake of serotonin [32]. The 5-HTTLPR S allele has been found associated with increased visceral sensitivity in IBS [33] and with depression and anxiety related traits [34,35].

Based on this information, it can be hypothesised that polymorphisms in HTR3A and 5-HTT genes might influence the sensory processes in the upper GI tract and affect dyspeptic symptom generation and reporting. In the present study we aimed to investigate the association between functional polymorphisms in these genes and dyspeptic symptom severity in primary care patients with uninvestigated dyspepsia. This association was studied in the knowledge that psychosocial comorbidity, IBS and coping styles should be included as potential confounders.

**Methods**

**Study population**

We performed a cross-sectional analysis of patients consulting with dyspepsia included in a large multicenter randomised treatment trial in primary care (DIAMOND trial). All patients included were consulting their General Practitioner with a new episode of dyspepsia, without alarm symptoms. They represent patients with dyspepsia managed in primary care. Details of the study design have been described elsewhere [36]. The study has been approved by the Medical Ethics Committees of the University Medical Centres Utrecht, Maastricht and Nijmegen.

Patients were enrolled after giving written informed consent. All data used for this study were registered at inclusion, before starting dyspepsia treatment. Self-reported questionnaires regarding gastrointestinal symptoms, demographic data (age, gender and ethnicity) psychopathology, life style factors; current smoker (yes/no) and current alcohol consumption (yes/no), use of medications, Irritable bowel syndrome (IBS) status (self reported; yes/no) were obtained at baseline. One blood sample was drawn for DNA extraction and determination of genotypes.

Dyspeptic symptoms were classified with a dyspepsia symptom questionnaire, validated by Veldhuyzen van Zanten [37]. It covers eight essential dyspeptic symptoms: epigastric pain, belching, heartburn, bloating, flatulence, regurgitation, nausea and halitosis. Severity of symptoms was registered on a 7 point Likert scale graded: (0) none, (1) minimal, (2) mild, (3) moderate, (4) moderately severe, (5) severe, (6) very severe. The symptom severity score is calculated by the sum of all items (range 0-48). Patients were classified as having mild, moderate and severe symptoms based on tertiles in the mean symptom score. Severe dyspeptic symptoms were defined as score ≥20.

Psychological problems were assessed using a validated Dutch version of SCL-90 questionnaire consisting of...
90 questions about 9 dimensions of psychological state [38]. In this analysis we used the SCL-90 dimension “psycho-neuroticism” which summarizes psychic dysfunction (calculated as a sum of all questions divided by the number of dimensions).

Coping styles were measured by a short version of the Utrecht Coping Questionnaire consisting of 17 items [39]. Six coping styles are distinguished, classified as: active coping, seeking support, avoidance coping, palliative coping, religious coping and passive reaction. Coping styles were rated on a four-point Likert scale ranging from (1) seldom or never, (2) sometimes, (3) often and (4) very often. Scale scores are the sums of the individual items. Higher scores indicate that the specific coping style is more often adopted.

Genotyping
Genomic DNA was extracted from whole blood using the QIAamp DNA blood minikit (Qiagen, Hilden, Germany). Genotyping of the HTR3A c.-42C > T polymorphism (rs1062613) was performed by Molecular Beacon assay using the iCycler iQ real-time PCR detection system (BioRad, Hercules, CA, USA). The assay was carried out in a total volume of 25 μl containing 50 ng of genomic DNA, 12.5 μl 2x iQ Supermix (BioRad, Hercules, CA, USA), 100 nM of forward primer (5’-GCGAAGCTCA-GAAGGTTG-3’), 250 nM of reverse primer (5’- CAGTTGAAGTGCTGTAGCC-3’) and 400 nM of each molecular beacon. MgCl2 was added to obtain a final concentration of 4 mM. Sequences of the molecular beacons were 5’-FAM-CGGACCAGTGTCAAGGCGAGGCGGT CCG-DABCYL-3’ (C-allele specific) and 5’-TXR-CGC GACCGAGTCTCAAGGAGCTGTCGTACG-DABCYL-3’ (T-allele specific). The PCR thermal cycling protocol applied consisted of an initial denaturation and enzyme activation step of 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 60°C for 1 min and 72°C for 45 s. In each run several controls were included: a “no template” control to check for contamination of reagents and positive controls for all three genotypes. To validate genotyping of HTR3A c.-42C > T by molecular beacon assay, sequencing was performed in a set of randomly chosen patients; concordance was 100%.

Genotyping of 5-HTTLPR polymorphism was performed by PCR and subsequent agarose gel electrophoresis. PCR was performed using the primers described by Camilleri et al [40]. The assay was carried out in a total volume of 25 μl, containing 50 ng of genomic DNA, 12.5 μl GC buffer I, 4.0 μl dNTP mix (2.5 mM each), 200 nM of each primer and 0.25 μl TaKaRa LA Taq polymerase (5 U/μl). The PCR thermal cycling protocol applied consisted of an initial denaturation step of 94°C for 1 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 2 min and a final extension step at 72°C for 5 min. The size of the amplified fragments was determined by electrophoresis on a 2.5% low range ultra agarose gel (Biorad, Hercules, CA, USA) stained with ethidium bromide; 572 bp and 528 bp products were typed as long (L) and short (S) alleles respectively.

Data analysis
Severity of dyspeptic symptoms was dichotomised as a sum score of ≥20 yes/no. Age was categorised as < 45 and ≥ 45 years.

The genotype distributions for the HTR3A c.-42C > T and 5-HTTLPR polymorphisms were tested for Hardy-Weinberg equilibrium using the Chi square test. In vitro studies have revealed that both the heterozygous (LS) and homozygous S genotypes of 5-HTTLPR result in reduced 5-HTT protein expression and uptake of serotonin [32]. Therefore, for 5-HTTLPR S allele carriers were analyzed versus subjects with the LL genotype. The significant difference in amygdaloidal activity in subjects with CC and CT genotypes of HTR3A c.-42C > T suggests a dominant effect of the T allele [41]. Therefore, we have analysed the CC genotype versus the combined homozygous and heterozygous T genotype.

Chi square test was used to test differences in genotype distributions, demographic, lifestyle and biologic factors, between patients with severe dyspepsia and mild and moderate dyspepsia. To assess association between genotype and phenotype logistic regression model with severe dyspeptic symptoms (yes/no) as dependent variable was used. For this model adjustments were made for age, IBS status, psycho neuroticism, use of antidepressants, use of acid suppressive medication and active coping style. Confounding effect by gender, psycho neuroticism, 5-HTTLPR genotype, alcohol use and smoking status was evaluated using stratified analyses.

To avoid bias due to race related differences in genotype distribution we excluded non Caucasian patients from the analysis. To prevent bias from missing values (4-15%) due to full or partial non response, regression method was used to impute missing values on the items of SCL-90, gastrointestinal symptom questionnaire, and other covariates.

All statistical analyses were performed with SPSS for Windows, version 14.0. P values less than the respective significance thresholds, obtained by applying Bonferroni correction for multiple testing, were considered statistically significant.

Results
From the 664 patients included in the DIAMOND study, 625 (94.1%) were of Caucasian origin. Blood samples for genotyping were obtained from 592 patients (94.7%). Between 20-30% of the patients graded their symptoms as mild to moderate (Table 1). Patients with severe
dyspepsia were younger (p < 0.05) and had higher level of psycho neuroticism (p < 0.05) than patients with mild and moderate dyspepsia (Table 2). No significant difference was observed regarding gender, smoking behaviour and IBS co morbidity, as well as alcohol consumption and co-medication use.

The genotype distributions of $HTR3A$ c.-42C > T and 5-HTTLPR were in concordance with Hardy-Weinberg equilibrium (Table 3). $HTR3A$ c.-42T allele was more prevalent among patients with severe dyspepsia (45.2 vs. 35.7%); the OR for association was 1.50 (95% CI 1.06-2.20). The association did not remain significant after Bonferroni correction for multiple testing (significance threshold $P = 0.025$). There was no association of 5-HTTLPR genotype considered as a single factor with dyspeptic symptom severity (Table 3).

To determine whether gender, 5-HTTLPR genotype, smoking and alcohol consumption and psycho neuroticism modify the effect of $HTR3A$ c.-42C > T genotype on dyspeptic symptoms we stratified for these factors. A significantly increased risk was found in females (OR 2.05, 95% CI 1.25-3.39) and in patients with 5-HTTLPR LL genotype (OR 2.00, 95% CI 1.01-3.94) (Table 4). The influence of female gender remained significant after

| Symptom          | Absent | Minimal | Mild | Moderate | Moderately Severe | Severe | Very Severe |
|------------------|--------|---------|------|----------|-------------------|--------|-------------|
| Epigastric pain  | 14.3   | 11.1    | 26.5 | 30.4     | 13.9              | 3.2    | 0.6         |
| Heartburn        | 16.0   | 12.3    | 19.4 | 22.7     | 20.1              | 6.3    | 3.2         |
| Regurgitation    | 17.2   | 14.1    | 25.3 | 21.5     | 15.1              | 4.7    | 2.1         |
| Nausea           | 35.8   | 22.3    | 22.1 | 9.2      | 6.6               | 2.9    | 1.1         |
| Bloating         | 14.2   | 13.8    | 23.2 | 21.7     | 19.0              | 6.2    | 2.0         |
| Belching         | 12.9   | 16.7    | 25.3 | 21.4     | 15.7              | 5.6    | 2.4         |
| Flatulence       | 10.2   | 17.8    | 30.7 | 22.0     | 13.7              | 4.5    | 1.1         |
| Halitosis        | 42.0   | 25.6    | 16.4 | 8.6      | 3.6               | 2.4    | 1.4         |

Table 1 Overview and grading of dyspeptic symptoms (%) in the study population

| Severe dyspeptic symptoms | Yes | No |
|---------------------------|-----|----|
| Number                    | 197 (33.3) | 395 (66.7) |
| Age, years                |     |    |
| < 45                      | 108 (54.8) | 151 (38.2) |
| ≥ 45                      | 89 (45.2)  | 244 (61.8) |
| Gender                    |     |    |
| Male                      | 84 (42.6)  | 186 (47.1) |
| Female                    | 113 (57.4) | 209 (52.9) |
| Current alcohol use       |     |    |
| Yes                       | 149 (75.6) | 295 (74.7) |
| No                        | 48 (24.4)  | 100 (25.3) |
| Current smoking           |     |    |
| Yes                       | 59 (29.9)  | 101 (25.6) |
| No                        | 138 (70.1) | 294 (74.4) |
| IBS                       |     |    |
| Yes                       | 10 (5.1)   | 17 (4.3) |
| No                        | 187 (94.9) | 378 (95.7) |
| Co-medication             |     |    |
| No                        | 123 (62.4) | 265 (67.0) |
| Antacids                  | 47 (23.9)  | 68 (17.2) |
| NSAIDs                    | 24 (12.2)  | 65 (16.4) |
| Antidepressants           | 17 (8.6)   | 19 (4.8) |
| Psycho neuroticism        |     |    |
| Yes                       | 95 (48.2)  | 107 (27.1) |
| No                        | 102 (51.8) | 288 (72.9) |
| Coping style              |     |    |
| Active coping             | 13.9 | 13.3 |
| Seeking support           | 11.7 | 11.1 |
| Avoidance coping          | 3.9  | 3.7 |
| Palliative coping         | 4.7  | 4.4 |
| Religious coping          | 3.2  | 3.0 |
| Passive reaction          | 2.0  | 2.0 |

Table 2 Patients characteristics according to dyspeptic symptom severity

Data presented as number (%) except for coping style, which values represent mean scores.
Bonferroni correction for multiple testing, whereas the 5-HTTLPR LL effect did not (significance threshold P = 0.0125). The additive effect of homozygous L 5-HTTLPR genotype appeared to be more pronounced in females (OR 3.50, 95% CI 1.37-8.90) (Table 5), which was also significant after Bonferroni correction for multiple testing (significance threshold P = 0.0125).

### Discussion

The results of this study suggest that patients carrying the HTR3A c.-42T allele are at increased risk of having severe dyspeptic symptoms. This risk seems to be even higher for women and patients homozygous for the 5-HTTLPR L allele.

The association could be explained as follows: Noxious and non-noxious visceral sensations are carried by extrinsic primary afferents to the dorsal horn of the spinal cord. Sensory transmission in the spinal dorsal horn is attenuated by endogenous inhibitory systems that originate at the brainstem. One of the main descending systems to the spinal dorsal horn is serotonergic [42]. 5-HT3 receptors present on spinal inhibitory interneurons receive input from the descending serotonergic fibers. Activation of these 5-HT3 receptors evokes release of GABA, which in turn reduces the excitability of dorsal horn neurons [43]. Consequently, the output of visceral sensory information to the brainstem and thereby symptom perception is reduced. It has been demonstrated in a model of visceral pain that 5-HT3 receptors in the spinal cord mediate antinociception [44]. The HTR3A c.-42T allele promotes translation of the HTR3A transcript resulting in enhanced production of the 5-HT3A subunit [27,28]. In vitro experiments indicate that homomeric 5-HT3A receptors have lower affinity for 5-HT and desensitize more rapidly as compared to heteromeric 5-HT3AB receptors [45]. Consistently, it has been found in 5-HTT knockout mice, in which 5-HT availability at the receptor is enhanced, that the expression of 5-HT3 subunits is altered, apparently leading to a relatively increased proportion of homomeric 5-HT3A receptors [46]. Thus, the increased expression of 5-HT3A subunits in HTR3A c.-42T allele carriers may result in a higher proportion of homomeric 5-HT3A receptors and as a consequence decreased response to 5-HT of the 5-HT3 receptor.

### Tables

#### Table 3: Association of HTR3A c.-42C > T and 5-HTTLPR genotypes with dyspeptic symptom severity

| Polymorphism | Genotype | Total N = 592 | Severe dyspeptic symptoms | OR (95% CI) * | P value |
|--------------|----------|---------------|---------------------------|---------------|---------|
|              |          | Yes N = 197   | No N = 395                |               |         |
| HTR3A c.-42C>T | CC       | 362 (61.1)    | 108 (54.8)                | 254 (64.3)    | 1.00    | 0.027  |
|              | CT       | 200 (33.8)    |                          |               |         |
|              | TT       | 30 (5.1)      |                          |               |         |
|              | CT + TT  | 230 (38.9)    | 89 (45.2)                 | 141 (35.7)    | 1.50    | 1.06-2.20 |
| 5-HTTLPR     | LL       | 170 (28.7)    | 59 (29.9)                 | 111 (28.1)    | 1.00    | 0.60   |
|              | LS       | 310 (52.4)    |                          |               |         |
|              | SS       | 112 (18.9)    |                          |               |         |
|              | LS + SS  | 422 (71.2)    | 138 (70.1)                | 284 (71.9)    | 0.90    | 0.60-1.40 |

Genotype distributions are depicted as number (%).

*adjusted for age, IBS status, psycho neuroticism, use of anti depressive and acid suppressive medication and active coping style.

#### Table 4: Association between severe dyspeptic symptoms and HTR3A c.-42C > T genotype stratified by gender and 5-HTTLPR genotype

| Gender | Severe dyspeptic symptoms | HTR3A c.-42C>T | OR (95% CI) * | P value |
|--------|---------------------------|----------------|---------------|---------|
|        |                           | CC             | CT + TT       |         |
| Female | Yes                       | 56 (49.6)      | 57 (50.4)     | 2.05    | 1.25-3.39 | 0.005 |
|        | No                        | 137 (65.6)     | 72 (34.4)     |         |           |       |
| Male   | Yes                       | 52 (61.9)      | 32 (38.1)     |         | 0.86     |       |
|        | No                        | 117 (62.9)     | 69 (37.1)     | 1.05    | 0.60-1.84 |       |
| 5-HTTLPR |                   |               |               |         |
| LL     | Yes                       | 31 (52.5)      | 28 (47.5)     | 2.00    | 1.01-3.94 | 0.046 |
|        | No                        | 77 (69.4)      | 34 (30.6)     |         |           |       |
| LS + SS | Yes                      | 77 (55.8)      | 61 (44.2)     |         | 0.15     |       |
|        | No                        | 177 (62.3)     | 107 (37.7)    | 1.38    | 0.88-2.15 |       |

Genotype distributions are depicted as number (%).

*adjusted for age, IBS status, psycho neuroticism, use of anti depressive and acid suppressive medication and active coping style.
involved in the descending antinociceptive pathway reflected in higher symptom severity. The additive effect of the LL genotype of the 5-HTTLPR polymorphism is conceivable as homozygosity for the long allele results in more rapid re-uptake of 5-HT and earlier termination of 5-HT induced signalling. As a consequence activation of the 5-HT3 receptor on inhibitory interneurons in the spinal cord is even more diminished; this reduces antinociception.

The association between severe dyspeptic symptoms and the \textit{HTR3A} \texttt{c.-42C > T} genotype appears to be stronger in females than in males. A possible explanation for this finding would be different availability of serotonin in males and females. It has been shown that ovarian hormones regulate the expression of tryptophan hydroxylase (rate-limiting enzyme of serotonin synthesis), 5-HTT and inhibitory 5-HT1A autoreceptors [47]. Lack of control for the variations in female sex hormones during the menstrual cycle, use of hormone replacement therapy and hormonal contraception has likely confounded many of the reported findings of gender differences in indices of serotonin availability. Interestingly, an \textit{in vivo} investigation with positron emission tomography and \textit{α}-[\textit{11C}]methyl-L-tryptophan trapping as a proxy for brain serotonin synthesis, showed a lower rate in healthy women compared to men [48]. Although the pre-menopausal women were studied in the same phase of the menstrual cycle, post-menopausal women were also included. The latter may have affected the extent of the reported difference in men and women. Moreover, this finding in healthy subjects does not necessarily reflect the situation in individuals with dyspepsia. Anyhow, a lower rate of serotonin synthesis in women coincides with a lower level of 5-HT available for receptor activation in females, which is consistent with reduced 5-HT3 receptor mediated antinociceptive effects.

In short, the presence of a 5-HT3 receptor with lower response to 5-HT due to polymorphism, rapid 5-HT re-uptake by 5-HTT and less serotonin available for receptor activation due to gender differences in 5-HT synthesis predisposes to increased perception of visceral stimuli. The influence of the \textit{HTR3A} \texttt{c.-42C > T} genotype has also been evaluated in (female) patients with irritable bowel syndrome (IBS) [30]. In contrast to what one might expect, since both dyspeptic and IBS symptoms have a visceral sensitivity component, the CC genotype appeared to be associated with greater IBS severity. Enhanced activity of amygdala-related emotional arousal circuits has been implicated in the pathophysiology of IBS [49]. Consistent with a study in healthy subjects, in IBS CC genotype subjects showed increased amygdala responsiveness to emotional facial stimuli compared with T carriers [30,41]. Activation of 5-HT3 receptors on GABAergic interneurons innervating the amygdala exerts an inhibitory influence on amygdala reactivity, whereas those on excitatory interneurons have the opposite effect [50]. The finding of lower amygdala reactivity in T carriers was interpreted as T-allele-related increased expression of 5-HT3 receptors on GABAergic interneurons resulting in greater inhibition of the amygdala [30,41]. These associations and interpretation of increased 5-HT3A subunit expression appear in conflict with our findings, but 5-HT3 receptor subunit composition may vary in the different regions of the central nervous system as well as the influence of 5-HT3 receptors on excitatory interneurons. Moreover, in dyspepsia increased amygdala responsiveness may be secondary to decreased spinal antinociception. It is noteworthy that another study in patients with IBS suggests that the \textit{HTR3A} \texttt{c.-42T} allele is associated with the diarrhea-predominant phenotype of the disease [28]. Patients with this phenotype seem to be more often affected by visceral hypersensitivity [51]. Furthermore, the \textit{HTR3A} \texttt{c.-42T} allele was found to be associated with visceral hypersensitivity in patients with gastroesophageal reflux disease [52].

### Table 5 Association between severe dyspeptic symptoms and \textit{HTR3A} \texttt{c.-42C > T} genotype stratified by 5-HTTLPR genotype in females and males

| Gender | 5-HTTLPR | Severe dyspeptic symptoms | \textit{HTR3A} \texttt{c.-42C > T} | OR (95% CI) | P value |
|--------|----------|--------------------------|-----------------------------|-------------|---------|
|        |          |                          | CC | CT + TT |          |         |
| Female | LL       | Yes                      | 16 (45.7) | 19 (54.3) | 3.50 (1.37-8.90) | 0.009  |
|        | No       |                          | 45 (75.0) | 15 (25.0)  |          |         |
|        | LS + SS  | Yes                      | 40 (51.3) | 38 (48.7)  | 0.090  |         |
|        | No       |                          | 92 (61.7) | 57 (38.3)  | 1.70 (0.93-3.14) |         |
| Male   | LL       | Yes                      | 15 (62.5) | 9 (37.5)   | 0.96 (0.32-2.89) | 0.94  |
|        | No       |                          | 32 (63.0) | 19 (37.0)  |          |         |
|        | LS + SS  | Yes                      | 37 (62.0) | 23 (38.0)  | 0.66  |         |
|        | No       |                          | 85 (63.0) | 50 (37.0)  | 1.16 (0.59-2.28) |         |

Genotype distributions are depicted as number (%).

*adjusted for age, IBS status, psycho neuroticism, use of anti depressive and acid suppressive medication and active coping style

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The HTR3A c.-42C > T polymorphism likely represents a predisposing genetic variant in common to psychiatric disorders and dyspepsia. Recently, effects of the HTR3A c.-42C > T genotype on emotional brain correlates of susceptibility to depression have been reported [53]. Furthermore, in healthy subjects and patients with IBS the CC genotype was found to be associated with greater anxiety ratings [30] and in Caucasians the C-allele was associated with elevated scores for the anxiety-related trait harm avoidance [29]. Although anxiety is comorbid with dyspepsia, our findings indicate that in dyspepsia the opposite allele is associated with greater overall symptom severity. Additional research is needed to directly examine the relationship between HTR3A c.-42C > T polymorphism and anxiety in patients with dyspepsia.

To appreciate results of this study several limitations should be mentioned. To date, most studies on 5-HT3 receptor composition and function have been performed in vitro or in rodents, which lack expression of the HTR3C, D, and E subunits [26]. It is not known yet how native receptors in humans are composed, including the 5-HT3 receptors on spinal inhibitory interneurons. Also the consequences of increased expression of 5-HT3A subunits for receptor composition and antinociceptive function have not been assessed in vivo. Secondly, there is a possibility that association between HTR3A c.-42T allele and severe dyspeptic symptoms is due to the effect of some other polymorphism, even in another gene, which is in linkage disequilibrium with HTR3A c.-42C > T. Indeed, the serotonin receptor subunit gene HTR3B maps in close proximity to HTR3A and the possibility has been raised that the HTR3A c.-42C > T polymorphism is not the susceptibility variant but a common variant in HTR3B, found to be associated with depression in Japanese [54]. Furthermore, we could not discriminate between patients with organic and functional dyspepsia. This could influence the results if the association would be specific only for one of them. In this respect it has to be mentioned that previously no significant association of HTR3A c.-42C > T and 5-HTTLPR polymorphisms with dyspeptic symptoms was detected in tertiary referral patients [17]. In the latter study organic disease was excluded, but we believe that the discrepant results can be explained by taking into account not merely the presence but also the severity of dyspeptic symptoms in the current study.

Conclusions
The results of this study suggest that there is an association between HTR3A c.-42T allele and severe dyspeptic symptoms. Altered 5-HT3 receptor function alone or in combination with 5-HTTLPR genotype could explain symptom severity in a subgroup of patients. The associations may be explained by the increased susceptibility to visceral hypersensitivity of the gastrointestinal tract and/or increased risk of having psychiatric comorbidity. Further research will have to replicate this result and clarify the clinical consequences of it.

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Authors’ contributions
SM, NW, CJM, GAIF, JJML, MEN designed the study. SM, CJM, and GAIF obtained the blood samples and questionnaire data. Laboratory work was undertaken by SM and JJML. SM, JJML, NW, DEG, and MEN analyzed the data and wrote the first draft of the paper. SM and CO carried out the statistical analysis. All authors contributed to and approved the final version of the manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Van Bommel MJ, Numans ME, de Wit NJ, Stalman WA: Consultations and referrals for dyspepsia in general practice—a one year database survey. Postgrad Med J 2001, 77:514-8.
2. Maconi G, Tosetti C, Stanghellini V, Bianchi Porro G, Corinaldesi R: Dyspeptic symptoms in primary care. An observational study in general practice. Eur J Gastroenterol Hepatol 2003, 14:985-90.
3. Meineche-Schmidt V, Krag E: Dyspepsia in general practice in Denmark. A 1-year analysis of consultants in general practice. Scand J Prim Health Care 1998, 16:216-21.
4. El-Serag HB, Talley NJ: Health-related quality of life in functional dyspepsia. Aliment Pharmacol Ther 2003, 18:387-93.
5. Halder SL, Locke GR, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ: Impact of functional gastrointestinal disorders on health-related quality of life: a population-based case-control study. Aliment Pharmacol Ther 2004, 19:233-42.
6. Tack J, Pesseaux H, Couille B, Caenepeel P, Janssens J: Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998, 115:1346-52.
7. Kim DY, Delgado-Arias S, Camilleri M, Samsom M, Murray JA, O’Connor MK, Brinkmann BH, Stephens DA, Lighvani SS, Burton DD: Noninvasive measurement of gastric accommodation in patients with idiopathic nonulcer dyspepsia. Am J Gastroenterol 2001, 96:3099-3105.
8. Sarrelli G, Caenepeel P, Gysens B, Janssens J, Tack J: Symptoms associated with impaired gastric emptying of solids and liquids in functional dyspepsia. Am J Gastroenterol 2001, 96:783-8.
9. Tack J, Caenepeel P, Fischer B, Pesseaux H, Janssens J: Symptoms associated with hypersensitivity to gastric distension in functional dyspepsia. Gastroenterology 2001, 121:526-35.
21. Tack J, Sarnelli G:
20. Kendler KS, Gardner CO, Gatz M, Pedersen NL:
19. Tahara T, Shibata T, Wang F, Yamashita H, Hirata I, Arisawa T:
18. Tahara T, Shibata T, Yamashita H, Hirata I, Arisawa T:
17. Van Lelyveld N, ter Linde JJM, Schipper M, Samsom M:

Functional dyspepsia: Are psychosocial factors of
25. Rajkumar R, Mahesh R:
The auspicious role of the 5-HT3 receptor in
26. Walstab J, Rappold G, Niesler B:
28. Kapeller J, Houghton LA, Mönnikes H, Walstab J, Möller D, Bönisch H,
29. The sources of co-morbidity between major depression and generalized anxiety disorder in a
27. Tahara T, Shhiba T, Wang F, Yamashita H, Hirata I, Arisawa T:
Genetic polymorphisms of molecules associated with innate immune responses, TL2R and MBL2 genes in Japanese subjects with functional dyspepsia.
Clin J Gastroenterol 2010, 47:217-23.

20. Kendler KS, Gardner CO, Gatz M, Pedersen NL: The sources of co-morbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. Psychological Medicine 2001, 31:453-62.

21. Tack J, Sarnelli G: Serotonergic modulation of visceral sensation: upper gastrointestinal tract. Gastroenterology 2006, 131:181-92.

22. van Oudenhove L, Vandenberghe J, Geeraerts B, Vos R, Persoons P,
23. Feinle C, Read NW:
Ondansetron reduces nausea induced by
24. Rhee PL, Kim YH, Son HJ, Kim JJ, Koh KC, Paik SW, Rhee JC, Choi KW:
Evaluation of individual symptoms cannot predict presence of gastric hypersensitivity in functional dyspepsia. Dig Dis Sci 2000, 45:1680-4.

25. Rajkumar R, Mahesh R: The auspicious role of the 5-HT3 receptor in
26. Walstab J, Rappold G, Niesler B: The role of the 5-HT3 receptor in functional disorders. Pharmacol Ther 2010, 128:146-69.

27. Niesler B, Rohr T, Noden MM, Fischer C, Rietschel M, Franken E, Albus M,
Propping P, Rappold GA: Association between the 5′UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics 2001, 11:471-5.

28. Kapeller J, Houghton LA, Mönnikes H, Walstab J, Möller D, Bönisch H,
29. The sources of co-morbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. Psychological Medicine 2001, 31:453-62.

30. Kilpatrick LA, Labus JS, Coveleskie K, Hammer C, Rappold G, Tillisch K,
31. Heils A, Teufel A, Mutzler M, Stöber G, Riederer P, Bengel D, Lesch KP: Alleric variation of human serotonin transporter gene expression. J Neurochem 1996, 66:2621-4.

32. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J,
Müller CK, Hamer DH, Murphy DL: Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996, 274:1527-31.

33. Camilli M, Busciglio I, Carlson P, McKinzie S, Burton D, Baxter K, Ryms K, Zemniemeier AR: Candidate genes and sensory functions in health and irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2008, 295:219-25.

34. Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B: The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. Arch Gen Psychiatry 2005, 62:229-35.

35. Sen S, Burmeister M, Ghosh D: Meta-analysis of the association between the serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. Am J Med Genet B Neuropsychiatr Genet 2004, 127:85-9.

36. van Marrewijk Cj, Muijukovia S, Fransen GA, Numans ME, de Witt NJ,
Muns VN, van Oijen MG, Jansen JB, Gribbee CE, Koortneers JA, Laihej RJ: Effect and cost-effectiveness of step-up versus step-down treatment with antacids, H2-receptor antagonists, and proton pump inhibitors in patients with new onset dyspepsia (DIAMOND study): a primary-care-based randomised controlled trial. Lancet 2009, 373:215-25.

37. Junghard G, Laursen K, Talley NJ, Wikkund IK: Validation of seven graded diary cards for severity of dyspeptic symptoms in patients with non-ulcer dyspepsia. Eur J Surg Suppl 1999, S33:161-163.

38. Arndell WA, Ettema JHM: SCL-90. Symptom checklist. Handleiding bij een multidimensionele psychopathologie-indicator. Swets Test Publishers, Lisse, 2003.

39. Schreurs PJG, van de Willige G, van de Brosshot JF, Tellegen B, Graus GMH:
De Utrechtse Coping Lijst (Utrecht Coping Questionnaire). Swets and Zeitlinger, Lisse, 1993.

40. Arrindell WA, Ettema JHM: Pharamaco genetics in diarrhea-predominant irritable bowel syndrome. Gastroenterology 2002, 123:425-32.

41. Ikeda T, Ozaki N, Matsumoto A, Nogawa J, Kinoshita Y, Suzuki T, Iwata N,
Yamamoto Y, Odaka T, Sadato N: A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. Neurosci 2003, 25:6460-6.

42. Millan MJ: Descending control of pain. Prog Neurobiol 2002, 66:355-474.

43. Alhaidar AA, Lien SZ, Wilcox GL: Spinal 5-HT3 receptor-mediated antinociception: possible release of GABA. J Neurosci 1999, 19:8818-8.

44. Danscheink RM, Gehlert DR, Grieb JP: Evidence that spinal 5-HT1, 5-HT2 and 5-HT3 receptor subtypes modulate responses to noxious colorectal distension in the rat. Brain Res Mol Brain Res 1990, 5:2948-51.

45. Dubin AE, Huvare R, D’Andreia MR, Patel J, Zhu JY, Joy KC, Wilson SJ,
Galindo JE, Glass CA, Luo L, Jackson MR, Lobergen TW, Eldridge MG: The pharmacological and functional characteristics of the serotonin 5-HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. J Biol Chem 1999, 274:3079-810.

46. Liu MT, Rayport S, Jiang Y, Murphy DL, Gershon MD: Expression and function of 5-HT3 receptors in the enteric neurons of mice lacking the serotonin transporter. Am J Physiol Gastrointest Liver Physiol 2002, 283:1398-411.

47. McEwen BS: Invited review: estrogens effects on the brain: multiple sites and molecular mechanisms. J Appl Physiology 2001, 91:7285-801.

48. Sakai Y, Nishikawa M, Leyton M, Benkefia C, Young SN, Diksic M:
Association of a functional variant in the microRNA-510 target site of the serotonin 5-HT(3A) receptor with depression: possible evidence for a functional variant in the microRNA-510 target site of the serotonin receptor-type 3E gene within the diahrea predominant irritable bowel syndrome. J Mol Genet Med 2008, 2:79-77.

49. Milke J, Westberg L, Nilsson S, Landen M, Soderstrom H, Baghfrai F,
Rosmond R, Holm G, Bjornstrom P, Nilsson L-G, Adolfsson R, Eriksson E: A polymorphism in the serotonin receptor 3A (HTR3A) gene and its association with human amygdala responsiveness in patients with irritable bowel syndrome. Gastroenterology 2011, 140:1593-51.

50. Barnes NM, Hales TG, Lummis SC, Peters JA: The 5-HT3 receptor-the relationship between structure and function. Neuropharmacology 2009, 56:273-84.
51. Houghton LA: Evidence of abnormal rectal sensitivity in IBS. In Irritable Bowel Syndrome: Diagnosis and Treatment. Edited by: Camilleri M, Spiller R. London: WB Saunders; 2002:69-76.

52. de Vries D, ter Linde J, van Herwaarden M, Smout M, Samson M: Serotonin receptor 3A polymorphism C178T is associated with visceral hypersensitivity in GERD [abstract]. Gastroenterology 2007, 132:A276.

53. Gatt JM, Williams LM, Schofield PR, Dobson-Stone C, Paul RH, Grieve SM, Clark CR, Gordon E, Nemeroff CB: Impact of the HTR3A gene with early life trauma on emotional brain networks and depressed mood. Depress Anxiety 2010, 27:752-9.

54. Yamada K, Hattori E, Iwayama Y, Ohishi T, Ohba H, Toyota T, Takao H, Minabe Y, Nakatani N, Higuchi T, Detera-Wadleigh SD, Yoshikawa T: Distinguishable haplotype blocks in the HTR3A and HTR3B region in the Japanese reveal evidence of association of HTR3B with female major depression. Biol Psychiatry 2006, 60:192-201.

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