Norepinephrine and Serotonin Transporter Genes: Impact on Treatment Response in Depression

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Key Words

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Abstract

Background/Aims: The norepinephrine transporter (NET) and serotonin transporter (5-HTT) genes constitute promising candidate genes in major depression. Seven polymorphisms in the promoter, intronic and exonic region of the *NET* gene, as well as serotonin-transporter-linked promoter region (5-HTTLPR) and 5-HTT rs25531 polymorphisms were analyzed with respect to antidepressant treatment response with particular attention to gender effects and subtypes of melancholic or anxious depression. **Methods:** 252 unrelated Caucasian patients ($f = 142$; $m = 110$) with major depression were genotyped for NET and 5-HTT polymorphisms. Genotype effects on Hamilton Depression Rating Scale score changes over 6 weeks of antidepressant treatment were analyzed using analysis of covariance with repeated measures. **Results:** There was no effect of any of the 7 investigated NET,

or the two 5-HTT polymorphisms, on the overall treatment response. An additional $-CT$ insertion/deletion (ins/del) polymorphism (rs58532686), however, was significantly associated with melancholic depression, with a better response in 12 patients carrying the deletion. Stratification for anxious versus nonanxious depression revealed a significantly detrimental effect of the less active 5-HTTLPR S allele ($p = 0.007$) and 5-HTTLPR/5-HTT rs25531 haplotypes on treatment response in patients with anxious depression. **Conclusion:** The present findings do not support a major impact of the NET and 5-HTT genes on antidepressant treatment response in major depression per se. However, there might be an impact of a $-CT$ ins/del polymorphism in the enhancer domain of the NET gene on treatment response in melancholic depression, which remains to be functionally investigated in future studies. The observed significant influence of the 5-HTT gene variation on antidepressant treatment in anxious depression points to anxious depression as a potential diagnostic entity of its own, requiring specific diagnostic and therapeutic attention.

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Introduction

Major depressive disorder is one of the most common psychiatric disorders with an estimated lifetime prevalence of at least 10%. Women are affected approximately twice as often as men [1, 2]. Heritability estimates of major depression vary between 40 and 50% [3, 4].

Norepinephrine transporter (NET; SLC6A2; MIM *163970) and serotonin transporter (5-HTT; SLC6A4; MIM *182138) are involved in the active reuptake of norepinephrine and serotonin from the synaptic cleft. NET and 5-HTT are primary targets of antidepressive agents such as norepinephrine reuptake inhibitors (NRI), serotonin and norepinephrine reuptake inhibitors (SNRI) and selective serotonin reuptake inhibitors (SSRI), which are effectively used in the pharmacological treatment of a major depressive episode [5]. Thus, the NET and 5-HTT genes constitute promising *a priori* candidate genes in the investigation of the pathomechanism of major depression and the mediation of antidepressant treatment response in particular.

There is a variety of association studies on genetic variants in 5-HTT (chromosome 17q11.1–12) influencing antidepressant treatment response in major depression. A recent meta-analysis on 15 studies including data of 1,435 subjects points to an association of the less active S allele of serotonin-transporter-linked promoter region (5-HTTLPR) with remission and response rates [6]. Additionally, the presence of the low-expression 5-HTTLPR S allele was found to be a strong risk factor associated with an adverse effect burden in antidepressant pharmacotherapy, while there was no overall effect on treatment response in the study by Hu et al. [7].

Considerably fewer studies, however, have dealt with the role of the NET gene in the pathogenesis of major depression and/or antidepressant treatment response. The NET gene located on chromosome 16q12.2 consists of 14 exons and shows a high sequence homology with dopamine and 5-HTT genes [8–12]. NET gene variation has been investigated in major depression, but with contradictory results: Three studies in Caucasian as well as Han Chinese populations report no association [13–15], while two other studies discerned an association of the T(–182)C polymorphism (rs2242446) with the disorder in Asian samples [16, 17]. To date, there is only one pharmacogenetic study reporting a significantly better treatment response in carriers of the –182T allele of the T(–182)C polymorphism (rs2242446) as compared to C allele carriers in a Japanese sample of 91 patients with major depression treated with milnacipran [18].

In order to further elucidate the role of genetic variants in the two major transporter proteins of the noradrenergic and serotonergic system, a set of 7 NET gene variants as well as the 5-HTTLPR and 5-HTT rs25531 polymorphisms were analyzed with respect to their impact on antidepressant treatment response in a sample of 252 Caucasian patients with major depression. In an attempt to further extend the present knowledge of the NET and 5-HTT influence on antidepressant treatment response, particular attention was paid to effects potentially specific to gender and melancholic or anxious depression as clinically relevant phenotypes of depression, which have been suggested to be differentially influenced by genetic variants [19–21].

Patients and Methods

Subjects

252 unrelated Caucasian patients (males: $n = 110$, mean age 49.3 ± 14.1 years; females: $n = 142$, mean age 51.2 ± 15.5 years; $p = 0.318$) with major depression admitted for inpatient treatment with a Hamilton Depression Rating Scale (HAM-D) admission score of >10 and a treatment cycle of 6 weeks from baseline were consecutively recruited at the Department of Psychiatry, University of Münster, Germany, between 2004 and 2006. The DSM-IV diagnosis of major depression including the melancholic subtype was ascertained by using the Structured Clinical Interview for Axis I Disorders according to the criteria of the DSM-IV. Additionally, anxious depression was measured according to Fava et al. [22] [21-item HAM-D (HAM-D-21) anxiety/somatization factor >7] in the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) sample [23]. The sample contained 131 patients scoring on Structured Clinical Interview for DSM-IV Axis I Disorders item A7 (worthlessness/inappropriate guilt). Patients with bipolar disorder, schizoaffective disorders or comorbid substance abuse disorders, mental retardation, pregnancy, neurological and neurodegenerative disorders or other clinically unstable medical illnesses impairing psychiatric evaluation were not included in this analysis. Caucasian descent was ascertained by a self-reported Caucasian background of both parents. The present sample has previously been analyzed for an association between other candidate genes [e.g. catechol-O-methyltransferase, monoamine oxidase (MAO) A] and antidepressant treatment response [24, 25]. The study was approved by the local ethical committee, and written informed consent was obtained from each patient.

Assessment

The clinical course of depression was assessed using the HAM-D-21, the Clinical Global Impression (CGI) scale and the Global Assessment of Functioning (GAF) scale. While scores on the HAM-D-21 were obtained on a weekly basis, CGI and GAF scores were obtained at admission and discharge only. Adverse drug effects were monitored clinically, but were not systematically assessed using standardized scales. The clinical response to treatment was measured by the intraindividual changes of HAM-D-21 scores over the 6-week study period [24].

Fig. 1. Schematic genomic organization and position of selected variants of the NET gene. Exons are shown as rectangles (not to scale). The positions of the selected polymorphisms are marked with arrows. SNP1: rs35915, -17215A/T; SNP2: rs28386840, -3081A/T [26]; SNP3: rs unknown, -3083C/T; SNP4: rs168924, -1014A/G (promoter polymorphism; [27]); SNP5: rs2242446, -133T/C (promoter polymorphism; [14, 16–18]); SNP6: rs36017, 28257G/C [28]; SNP7: rs47958, 36001A/C [28]; SNP8: rs171798, T/C; -/CT ins/del polymorphism: rs58532686. Linkage disequilibrium (LD) analysis was estimated on the basis of the solid spine of LD (www.hapmap.org).

Color version available online

Medication

The patients were treated in a naturalistic setting with a variety of antidepressant medication (mirtazapine: $n = 28$, 11.1%; citalopram/escitalopram: $n = 38$, 15.1%; venlafaxine: $n = 45$, 17.9%; mirtazapine plus citalopram/escitalopram: $n = 38$, 15.1%; mirtazapine plus venlafaxine: $n = 61$, 24.2%; other medication, e.g. tricyclic antidepressants, NRI, MAO inhibitors or lithium: $n = 42$, 16.7%). As comedication, atypical antipsychotics (quetiapine, olanzapine, risperidone: $n = 120$, 47.6%) as well as mood stabilizers (lithium, valproic acid: $n = 58$, 23.0%) were used in addition to antidepressant treatment. Benzodiazepines were used in 3 cases only. None of the included patients had received electroconvulsive therapy in the 6 months before the present investigation.

Selection of Single-Nucleotide Polymorphisms and Genotyping

NET gene polymorphisms were selected based on in silico analyses using the HapMap database (<http://www.hapmap.org>) and the UCSC (University of California, Santa Cruz) human genome browser (<http://genome.ucsc.edu>) to cover most parts of the NET genomic region. In addition, potentially functionally relevant variants as well as polymorphisms reported in previous association studies were included (fig. 1): single-nucleotide polymorphism (SNP) 1 (rs35915, -17215A/T); SNP2 (rs28386840, -3081A/T) [26]; SNP3 (rs unknown, -3083C/T, not included in pharmacogenetic analysis since it is not polymorphic); SNP4 (rs168924, -1014A/G) [27]; SNP5 (rs2242446, -133T/C) [14, 16–18]; SNP6 (rs36017, 28257G/C) [28]; SNP7 (rs47958, 36001A/C) [28], and SNP8 (rs171798, T/C). Linkage disequilibrium (LD) analysis revealed, on the one hand, a high LD between the 4 NET SNPs rs28386840, rs unknown -3083C/T, rs168924 and

rs2242446, and on the other hand between the 3 NET SNPs rs36017, rs47958 and rs171798, which led to 2 haplotype blocks of 3 kb and 25 kb, respectively. The LD analysis was estimated on the basis of the solid spine of LD (fig. 1).

Genomic DNA was purified from the buffy coat of EDTA venous blood samples, using the FlexiGene DNA kit (Qiagen) according to the manufacturer's instructions. SNPs were genotyped by 5'-exonuclease TaqMan SNP genotyping assays (ABI Prism 7900 Sequence Detection System with SDS software version 2.1; Applied Biosystems, Darmstadt, Germany) and by PCR-based restriction fragment length polymorphism (RFLP) assays (table 1). For genotyping quality control, all TaqMan assays were validated by genotyping representative samples (28%) by RFLP, which yielded concordance rates of 100%, with the exception of NET SNP2 (rs28386840) where 12 discrepancies were detected. Thus, discrepant probes were additionally genotyped for rs28386840 by bidirectional sequencing on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). Results revealed a deletion as well as an additional, previously unreleased SNP (NET SNP3: position 54243921; UCSC) 2 bp downstream (3') to rs28386840, disrupting the cutting site of *Bse*NI.

Additionally, all patients were genotyped for 5-HTTLPR and 5-HTT rs25531 according to published protocols [29, 30] with minor modifications [31]. The genotypes were determined blind for phenotypes, and independently by 2 investigators.

Cloning and Sequencing

The PCR products were cloned into a 3'-T-overhang plasmid vector (TOPO TA Cloning; Invitrogen) using standard procedures. The PCR conditions and primer sequences are shown in table 1. Li-

Table 1. Genotyping conditions with primer sequences for the examined NET polymorphisms

SNP ID	Primer pair (5'–3')	Annealing temperature, °C	Amplicon size, bp	Restriction enzyme	Fragment size, bp
NET SNP1 rs35915	F: TTGTTTTCTGGCCAATCTTTCTC R: TGCTCAGAAATGCTCTTTCAAGT	60	467	<i>Eam</i> 1104I	A: 388, 32, 24, 23 T: 339, 49, 32, 24, 23
NET SNP2 rs28386840	F: CCCAATGGCTTCCTGCTGCCAGTAC R: TTGGGTTTGGTGTCTTTGATTGGTTTCG	68	418	<i>Bse</i> NI	A: 203, 138, 58, 19 T: 341, 58, 19
NET SNP3 C/T	F: CAGTACCTGGGGCTCTGCTGTTAGCCTTTCT R: AGTAGATTAAGGAGCACCTTGCTGCTGGGTAA	65	238	<i>Hpy</i> 8I	C: 150, 88 T: 120, 88, 30
NET SNP4 rs168924	F: TTTAAGTGGCCTGCTGCGGAAGAC R: CGAACGCCACACGGCGGAGC	61	470	<i>Alu</i> I	A: 404, 38, 28 G: 432, 38
NET SNP5 rs2242446	F: AAGTGTCGAGAGAAGGCTCCTG R: GAGCGCTTAGACACTGGGAT	60	302	<i>Bsl</i> I	T: 138, 115, 49 C: 115, 85, 52, 49, 1
NET SNP6 rs36017	F: TCTTCCCTCCTGCTGAGAACTGCT R: AGGGACCCTAGCCACTGCCAT	65	243	<i>Bst</i> NI	G: 99, 81, 63 C: 81, 63, 54, 45
NET SNP7 rs47958	F: CTAGTCCACCCAGCCATT R: CTGGACTCCACTACCAAATTCAC	62	210	<i>Bsp</i> LI	A: 167, 43 C: 124, 43, 43
NET SNP8 rs171798	F: TGGAATTGGAAGGACAACAAA R: AACTGACACAAGGTAAACCAACC	60	196	<i>Tru</i> II	T: 116, 44, 36 C: 160, 36
NET –/CT ins/del rs58532686	F: CCCAATGGCTTCCTGCTGCCAGTAC R: TTGGGTTTGGTGTCTTTGATTGGTTTCG	68	418	<i>Tai</i> I	CT-deletion: 224, 192 CT-insertion: 418

gations were transformed into competent *Escherichia coli* and plated onto kanamycin-containing plates. Colonies were picked and minipreps performed using standard procedures. Deletions were sequenced in both directions using dye terminator chemistry.

Statistical Analysis

Comparisons of HAM-D, Beck Depression Inventory (BDI), CGI and GAF baseline scores across the genotype groups were carried out by one-way analysis of covariance (ANCOVA; SPSS version 16.0). The HAM-D score change over 6 weeks of treatment was normally distributed as assessed by the Kolmogorov-Smirnov test. Homoscedasticity was verified by Levene's test for the homogeneity of variances. The pharmacogenetic investigation of NET and 5-HTT variant effects on HAM-D score change over 6 weeks of antidepressant treatment was performed using an ANCOVA with repeated measures, i.e. genotype as fixed factor, time point as repeated measure and a number of covariates (see below) with Bonferroni's correction for multiple comparisons (tables 2, 3). Bonferroni-corrected *p* values were computed as part of the ANCOVA procedure with 3 comparisons per SNP and analysis. Since Bonferroni's correction was applied as implemented in SPSS as part of the ANCOVA procedure, it was not possible to additionally correct for haplotype or depression subtype tests, which has to be considered as a limitation. In order to assure a most conservative report of data, only Bonferroni-corrected *p* values are given; raw *p* values can be obtained from the authors upon request. Variables such as age, gender, polypharmacy, treatment with antidepressants plus neuroleptics, duration of depressive illness, lifetime number of depressive episodes, number of hospitalizations due to depression, class of antidepressant (i.e. SSRI, serotonin- and norepinephrine-releasing agent, Na selective sero-

tonin-releasing agent, tricyclic antidepressants, MAO inhibitors and antidepressants plus mood stabilizer) or family history of psychiatric disorders were included as covariates if a significant effect on treatment response over 6 weeks was observed in univariate analyses. Stratified analyses for gender, melancholic depression and anxious depression were performed (tables 2, 3).

In addition, individually reconstructed haplotype alleles were assigned by PHASE, version 2.11 [32], based on a probability of reconstructed pairs of haplotypes for each individual of >0.99. The 6 most frequent haplotypes of the blocks encompassing SNPs 2, 3, 4 and 5 (rs28386840, rs unknown, rs168924 and rs2242446) and SNPs 6, 7 and 8 (rs36017, rs47958 and rs171798) were included in subsequent analyses (table 4).

Power calculations were carried out applying the software tool Quanto, version 1.2.3 (<http://hydra.usc.edu/GxE/>). The sample had a power of 80% to detect a proportion of variance, $R^2 = 0.05$, in HAM-D explained by genetic effects (*p* adjusted for 8 times multiple testing, $p < 0.00625$). Hardy-Weinberg equilibrium was examined using the program Finetti provided as an online source (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>; T.F. Wienker and T.M. Strom).

Results

In our sample of 252 patients with major depression, the subsamples stratified for gender, melancholic subtype of depression and comorbidity with anxiety did not significantly differ for age, education or marital status. The mean HAM-D score at admission was 23.1 ± 7.3 , and at

Table 2. Overall percent reduction in HAM-D scores across NET genotypes in patients with major depression (n = 252) during the 6-week course of antidepressant treatment

	Percent HAM-D reduction	Percent HAM-D reduction	Percent HAM-D reduction	p ^{1,2}
<i>NET SNP1 rs35915</i>				
HAM-D change weeks 2–6 ^{1,3}	AA	AT	TT	AA/AT vs. TT*
MDD (n = 252)	–9.9 ± 20.9 (3)	–29.1 ± 4.3 (72)	–25.8 ± 2.7 (177)	0.61
Females (n = 142) ²	–8.6 ± 25.8 (2)	–23.2 ± 5.4 (44)	–26.8 ± 3.7 (96)	0.52
Males (n = 110) ²	–3.4 ± 36.9 (1)	–38.7 ± 6.9 (28)	–24.7 ± 6.1 (81)	0.12
Anxious depression (n = 91)	–44.4 ± 39.1 (1)	–38.1 ± 5.2 (29)	–34.9 ± 7.5 (61)	0.76
Melancholic depression (n = 82)	–9.9 ± 20.9 (2)	–29.1 ± 4.3 (28)	–25.8 ± 2.7 (52)	0.55
<i>NET SNP2 rs28386840</i>				
HAM-D change weeks 2–6 ^{1,3}	TT	AT	AA	
MDD (n = 252)	–26.1 ± 8.6 (18)	–25.7 ± 3.9 (86)	–27.2 ± 2.9 (148)	0.76
Females (n = 142) ²	–31.6 ± 12.8 (8)	–28.4 ± 4.9 (53)	–22.8 ± 4.1 (81)	0.33
Males (n = 110) ²	–22.8 ± 11.7 (10)	–20.8 ± 6.5 (33)	–32.4 ± 4.5 (67)	0.13
Anxious depression (n = 91)	–20.7 ± 14.2 (10)	–39.3 ± 7.9 (29)	–38.5 ± 5.5 (52)	0.66
Melancholic depression (n = 82)	–43.9 ± 13.6 (7)	–26.2 ± 6.5 (29)	–27.9 ± 5.2 (46)	0.84
<i>NET –/CT deletion rs58532686</i>				
HAM-D change weeks 2–6 ^{1,3}	present		not present	
MDD (n = 252)	–36.9 ± 10.4 (12)		–26.1 ± 2.3 (240)	0.31
Females (n = 142) ²	–36.8 ± 13.7 (7)		–24.8 ± 3.1 (135)	0.39
Males (n = 110) ²	–37.3 ± 16.5 (5)		–27.7 ± 3.6 (105)	0.57
Anxious depression (n = 91)	–54.9 ± 39.1 (2)		–36.9 ± 4.2 (89)	0.65
Melancholic depression (n = 82)	–79.8 ± 19.6 (3)		–26.7 ± 3.8 (79)	0.01
<i>NET SNP4 rs168924</i>				
HAM-D change weeks 2–6 ^{1,3}	GG	AG	AA	GG/AG vs. AA*
MDD (n = 252)	–39.5 ± 25.9 (4)	–29.0 ± 4.2 (76)	–25.4 ± 2.8 (172)	0.44
Females (n = 142) ²	–70.2 ± 36.4 (2)	–21.8 ± 5.0 (50)	–26.9 ± 3.8 (90)	0.49
Males (n = 110) ²	–6.1 ± 36.5 (2)	–43.9 ± 7.5 (26)	–23.6 ± 4.0 (82)	0.02
Anxious depression (n = 91)	–	–42.1 ± 7.6 (28)	–34.9 ± 5.1 (63)	0.43
Melancholic Depression (n = 82)	–35.1 ± 25.9 (2)	–30.1 ± 6.7 (29)	–27.6 ± 5.0 (51)	0.73
<i>NET SNP5 rs2242446</i>				
HAM-D change week 2–6 ^{1,3}	CC	CT	TT	
MDD (n = 252)	–26.4 ± 8.6 (18)	–25.5 ± 3.8 (90)	–27.3 ± 3.0 (144)	0.72
Females (n = 142) ²	–31.5 ± 12.8 (8)	–26.9 ± 4.9 (54)	–23.7 ± 4.1 (80)	0.53
Males (n = 110) ²	–23.4 ± 11.9 (10)	–23.5 ± 6.3 (36)	–31.4 ± 4.6 (64)	0.28
Anxious depression (n = 91)	–35.4 ± 7.6 (10)	–40.9 ± 5.6 (31)	–20.8 ± 14.2 (50)	0.32
Melancholic depression (n = 82)	–42.4 ± 12.7 (8)	–26.6 ± 6.5 (29)	–27.6 ± 5.3 (45)	0.79
<i>NET SNP6 rs36017</i>				
HAM-D change weeks 2–6 ^{1,3}	GG	CG	CC	
MDD (n = 252)	–20.8 ± 4.8 (57)	–27.4 ± 3.2 (131)	–30.1 ± 4.5 (64)	0.37
Females (n = 142) ²	–24.4 ± 6.5 (31)	–24.3 ± 4.3 (71)	–28.1 ± 5.7 (40)	0.57
Males (n = 110) ²	–16.8 ± 7.3 (26)	–13.2 ± 4.8 (60)	–32.9 ± 7.6 (24)	0.48
Anxious depression (n = 91)	–27.6 ± 9.5 (21)	–36.8 ± 6.1 (43)	–43.7 ± 7.7 (27)	0.30
Melancholic depression (n = 82)	–21.9 ± 9.2 (15)	–28.4 ± 5.3 (45)	–33.8 ± 7.6 (22)	0.43
<i>NET SNP7 rs47958</i>				
HAM-D change weeks 2–6 ^{1,3}	AA	AC	CC	
MDD (n = 252)	–21.3 ± 4.8 (56)	–26.9 ± 3.2 (130)	–30.4 ± 4.5 (66)	0.32
Females (n = 142) ²	–25.6 ± 6.2 (30)	–23.8 ± 4.3 (71)	–28.1 ± 5.6 (41)	0.57
Males (n = 110) ²	–16.8 ± 7.3 (26)	–30.8 ± 4.9 (59)	–33.7 ± 7.4 (25)	0.38
Anxious depression (n = 91)	–27.6 ± 9.5 (21)	–36.4 ± 6.1 (41)	–44.1 ± 7.5 (29)	0.26
Melancholic depression (n = 82)	–23.3 ± 9.5 (14)	–27.9 ± 5.4 (45)	–33.5 ± 7.5 (23)	0.44

Table 2 (continued)

	Percent HAM-D reduction	Percent HAM-D reduction	Percent HAM-D reduction	p ^{1,2}
<i>NET SNP8 rs171798</i>				
HAM-D change weeks 2–6 ^{1,3}	CC	CT	TT	CC/CT vs. TT*
MDD (n = 252)	–29.7 ± 18.3 (4)	–27.5 ± 4.6 (62)	–26.3 ± 2.7 (186)	0.79
Females (n = 142) ²	–30.1 ± 18.1 (4)	–25.9 ± 6.1 (36)	–25.1 ± 3.6 (102)	0.85
Males (n = 110) ²	–	–29.1 ± 7.3 (26)	–27.8 ± 4.1 (84)	0.88
Anxious depression (n = 91)	–	–28.7 ± 8.8 (23)	–39.7 ± 4.8 (68)	0.28
Melancholic depression (n = 82)	29.8 ± 36.1 (1)	–21.7 ± 7.5 (23)	–31.4 ± 4.6 (58)	0.29

Values denote means ± SE with numbers in parentheses. SNP3 (rs unknown, –3083C/T) was not included in the pharmacogenetic analysis due to its low ‘polymorphicity’. p values: ANCOVA with repeated measures (covariates: age, gender, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics) and with post-hoc Bonferroni correction. p values in italics <0.05, Bonferroni-corrected for multiple comparisons. Polypharmacy: treatment with various antidepressants. * Genotypes were grouped in a binary variable in case one genotype had n < 5, so that this low-frequency genotype was combined with the heterozygous genotype and compared to the more frequent homozygous genotype. MDD = Major depressive disorder.

¹ p values from ANCOVA models with covariates age, gender, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics.

² p values from ANCOVA models with covariates age, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics.

³ Intraindividual relative change in scores of the HAM-D-21 scale between weeks 2 and 6. ANCOVA (covariates: age, gender, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics) with repeated measures independent of change in week 1.

discharge it was 6.0 ± 5.0 , without showing any differences between genders.

The genotype, allele and haplotype frequencies of the respective NET and 5-HTT polymorphisms are given in tables 2 and 3. The genotype distributions did not deviate from Hardy-Weinberg equilibrium. There were no significant differences in gender, subtypes of depression, overall and anxiety-specific HAM-D scores, BDI, GAF and CGI baseline scores between NET or 5-HTT genotypes in the overall sample as well as in the sample stratified for gender. The type of antidepressant (SSRI vs. others), antidepressant treatment alone (monotherapy vs. polypharmacy) or in combination with mood stabilizers (antidepressant alone vs. antidepressant plus mood stabilizer) or with neuroleptics (antidepressant alone vs. antidepressant plus atypical neuroleptics) as well as the historical number of depressive episodes (continuous variable), duration of illness and number of hospitalizations (both continuous variables) were not related to NET or 5-HTT genotypes. Since only age, gender, polypharmacy (treatment with various antidepressants), treatment with antidepressants plus atypical antipsychotics and HAM-D score at admission were related to treatment response over 6 weeks, these variables were considered as covariates in the subsequent ANCOVA.

NET Gene

Overall ANCOVA with repeated measures for the intraindividual percent HAM-D score reduction over the course of the 6 weeks of antidepressant treatment across the NET genotype groups did not reveal any impact of the investigated 7 SNPs on treatment response.

Interestingly, cloning and sequencing of an additional PCR fragment present in the RFLP analyses of 12 patients identified an ins/del polymorphism in the enhancer domain of the NET gene [26], which was nominally significantly associated with melancholic depression: 12 patients carrying the deletion showed a better treatment response as compared to 240 patients with the insertion (table 2). Cloning of the PCR products indicated this polymorphism to be a 2-base-pair deletion (CT). This deletion occurred in heterozygous form. The analysis of the sequenced colonies revealed a dinucleotide deletion (rs58532686) at position 54244318–54244319 consisting of the nucleotide C and the SNP rs28386840 as well as the original amplicon. The deletion was confirmed by RFLP assay in the 12 different specimens (table 1).

The haplotype analyses of the 6 most frequent haplotypes revealed no significant associations with treatment response (table 4). When analyzing the impact of the most frequent haplotypes on pharmacoresponse strati-

Table 3. Overall percent reduction in HAM-D scores across 5-HTTLPR genotypes and 5-HTTLPR/rs25531 haplotypes in patients with major depression (n = 252) stratified for gender as well as anxious and melancholic depression during the 6-week course of antidepressant treatment

	Percent HAM-D reduction	Percent HAM-D reduction	p ^{1,2}
<i>5-HTTLPR</i>			
HAM-D change weeks 2–6 ^{1,3}	LL (n = 91)	SL/SS (n = 161)	
MDD (n = 252)	–30.4 ± 3.8 (91)	–24.4 ± 2.9 (161)	0.22
Females (n = 142) ²	–28.1 ± 4.9 (52)	–23.9 ± 3.8 (90)	0.30
Males (n = 110) ²	–33.2 ± 6.0 (39)	–25.4 ± 4.4 (71)	0.50
Anxious depression (n = 91)	–52.7 ± 6.9 (30)	–28.9 ± 5.0 (61)	<i>0.007</i>
Melancholic depression (n = 82)	–31.6 ± 5.9 (36)	–26.3 ± 5.2 (46)	0.51
<i>5-HTTLPR/rs25531</i>			
HAM-D change weeks 2–6 ^{1,3}	L _A L _A	rest ⁴	
MDD (n = 252)	–28.7 ± 4.3 (73)	–25.7 ± 2.7 (179)	0.56
Females (n = 142) ²	–27.7 ± 5.5 (44)	–24.4 ± 3.6 (98)	0.61
Males (n = 110) ²	–29.4 ± 7.1 (29)	–27.7 ± 4.2 (81)	0.84
Anxious depression (n = 91)	–52.6 ± 8.8 (21)	32.4 ± 4.8 (70)	<i>0.049</i>
Melancholic depression (n = 82)	–28.5 ± 6.4 (31)	28.7 ± 4.9 (51)	0.97

Values denote means ± SE with numbers in parentheses. p values: ANCOVA with repeated measures (covariates: age, gender, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics) and with post-hoc Bonferroni correction. p values in italics <0.05, Bonferroni-corrected for multiple comparisons. Polypharmacy: treatment with various antidepressants. Polypharmacy: treatment with various antidepressants. MDD = Major depressive disorder; L = 5-HTTLPR long allele; S = 5-HTTLPR short allele.

¹ p values from ANCOVA models with covariates age, gender, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics.

² p values from ANCOVA models with covariates age, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics.

³ Intraindividual relative change in scores of the HAM-D-21 scale between weeks 2 and 6. Multivariable ANCOVA (covariates: age, gender, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics) with repeated measures independent of change in week 1.

⁴ Haplotypes L_AL_G, L_GL_G, S_AS_A, S_AS_G, S_GS_G, L_AS_A, L_AS_G, L_GS_A and L_GS_G.

fied for the class of antidepressants (SSRI, SNRI, NRI), no significant genetic effects were observed either.

5-HTT Gene

There was no significant influence of 5-HTTLPR on the intraindividual percent HAM-D score reduction over the course of the 6 weeks of antidepressant treatment in major depression either in the overall sample or the subsamples stratified for gender. Moreover, no association of the 5-HTTLPR genotype with melancholic depression as a specific subtype of the disease could be detected. However, stratification for anxious versus nonanxious depression revealed a significantly detrimental effect of the less active 5-HTTLPR S allele on antidepressant treatment response (p = 0.007) in patients with anxious depression (table 3; fig. 2).

Similar results were observed when including 5-HTT rs25531 in the analysis with the more active 5-

HTTLPR/5-HTT rs25531 haplotype L_AL_A conveying a significantly better response as compared to the less active 5-HTTLPR/5-HTT rs25531 haplotypes (rest; p = 0.049) (table 3).

Discussion

The present study does not suggest a role of a set of 7 NET gene variants in antidepressant treatment response in major depression, which is in line with previous findings regarding single NET polymorphisms [13–17]. It is, however, in contrast to a Japanese pharmacogenetic study reporting a significantly better treatment response in carriers of the –182T allele of the T(–182)C polymorphism (rs2242446) in patients with major depression treated with milnacipran [18]. A possible explanation for this discrepancy might be that Yoshida et al. [18] ana-

Fig. 2. Percent change in HAM-D score over 6 weeks depending on 5-HTTLPR genotype in patients with anxious depression (n = 91). Percent change in score derived from separate multivariable ANCOVA with covariates age, gender, polypharmacy, HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics. Error bars = SD.

Table 4. Overall percent reduction in HAM-D scores across NET haplotypes in patients with major depression (n = 252) during the 6-week course of antidepressant treatment

		Number	Percent HAM-D reduction		
			haplotype	vs. rest	p ¹
Haplotype SNPs 6, 7, 8					
GAT	240 (47.6%)	-25.8 ± 2.9	-28.9 ± 4.3	0.54	
CCT	191 (37.9%)	-27.6 ± 2.7	-25.0 ± 3.5	0.57	
CCC	68 (13.5%)	-26.3 ± 4.3	-26.7 ± 2.5	0.91	
GCT	3 (0.6%)	–	–	–	
GAC	2 (0.4%)	–	–	–	
Total	504 (100%)				
Haplotype SNPs 2, 3, 4, 5					
ATAT	296 (58.7%)	-26.5 ± 2.4	-27.3 ± 5.3	0.90	
TTAC	119 (23.7%)	-26.3 ± 3.4	-26.8 ± 2.8	0.89	
ATGT	79 (15.7%)	-29.7 ± 3.9	-25.2 ± 2.6	0.35	
ATAC	7 (1.3%)	–	–	–	
TCAT	2 (0.4%)	–	–	–	
TTGT	1 (0.2%)	–	–	–	
Total	504 (100%)				

¹ p value of intraindividual relative change in scores of the HAM-D-21 between weeks 2 and 6, between groups. ANCOVA with covariates age, gender, polypharmacy (treatment with various antidepressants), HAM-D score at admission and treatment with antidepressants plus atypical antipsychotics, and with repeated measures independent of change in week 1.

lyzed a sample of patients treated with milnacipran, an SNRI directly acting on the NET, while in the present sample, the patients were treated in a naturalistic setting with a variety of antidepressants, mainly serotonin reuptake inhibitors, which might have prevented a NET-spe-

cific finding as suggested by Kim et al. [33] in a sample of patients with late-life depression. However, a subanalysis of the patients treated with SNRI (n = 45) or NRI (n = 9) in the present sample did not reveal a significant impact of NET gene variation on treatment response. This might be due to the fact that SNRI such as venlafaxine are potent 5-HTT antagonists, but less potent NET antagonists in vivo [34], and thus might not primarily be dependent on NET gene variants. Also, NET inhibition by venlafaxine only occurs at higher doses (225 and 375 mg/day) [35], which could not be evaluated in the relatively small sample of patients treated with SNRI in the present study. Thus, further studies are warranted in order to elucidate a possible effect of NET gene variation on the treatment response to norepinephrine reuptake inhibition in particular. A potentially additional reason for the discrepant findings in the Japanese study [18] and in the present analysis might be an ethnically differential allele distribution (rs2242446 in Japanese population: CC 0.200; CT 0.422; TT 0.378; in European population: CC 0.033; CT 0.467; TT 0.500; NCBI dbSNP Build 129), an effect that has previously been described in other genetic association studies, e.g. regarding the catechol-O-methyltransferase Val158Met polymorphism in panic disorder [36].

Interestingly, a presently observed ins/del polymorphism which, in cloning experiments, was revealed to be a -/CT ins/del variant (rs58532686), was significantly associated with melancholic depression, with a better response in 12 patients carrying the deletion as compared to 240 patients with the insertion (table 2). Given the location of rs58532686 in the enhancer domain of the NET gene [26], this polymorphism might be of functional relevance with respect to the transcription of the gene. In silico analyses using online transcription factor binding site prediction tools (TRANSFAC 2008.3) revealed that

the 2-bp ins/del affects a transcription factor binding site. In the presence of the insertion, the transcription factors c-Myc, USF2, HNF-3 α and HNF-3B can potentially bind, while these binding sites are disrupted by the deletion, which only creates a binding site for HIF1. So far, these transcription factors have not been implicated in the noradrenergic system and, therefore, the functional relevance of the observed ins/del variant association remains to be elucidated in future studies.

Additionally, we discerned a significantly detrimental effect of the NET SNP4 rs168924 G allele on antidepressant treatment response in the male subgroup of the patients. This finding might point to a possible gender-specific role of NET gene variation, particularly since the feedback regulation effect of sex steroids on luteinizing hormone secretion has been shown to involve transcriptional/translational processes of NET in brainstem noradrenergic cells [37]. However, due to the small sample size of the male subgroup of patients carrying the rs168924 GG genotype, this finding might well be a false-positive one.

There was no overall influence of 5-HTT gene variation (5-HTTLPR and SNP rs25531) on treatment outcome in major depression, which is in line with STAR*D not reporting a pharmacogenetic effect for 5-HTT [7, 38]. However, a detrimental effect of the less active 5-HTTLPR S allele on antidepressant treatment response was detected in the diagnostic subgroup of patients with anxious depression. This is in line with anxious depression being specifically associated with poorer treatment response than nonanxious depression [22, 23, 39] as well as with previous findings of differential genetic effects in anxious versus nonanxious depression [23]. 5-HTT gene variation has particularly been implicated in the pathogenesis of both major depression [20] and anxiety disorders [40], as well as in the mediation of the response to SSRI treatment in generalized social anxiety disorder [41] and panic disorder [42]. Thus, 5-HTT gene variation might influence an intermediate phenotype common to major depression and anxiety disorders.

Some limitations have to be considered while interpreting the present results. Comorbidity with personality disorders could not be controlled for, which might have confounded the present finding. Recruitment in a naturalistic setting allowed for a large sample size; however, it implies treatment with a variety of antidepressants partly in combination with mood stabilizers and antipsychotics, no standardized dosage regime and no standardized control for plasma drug levels. Thus, treatment compliance could only be controlled for by routine nurse obser-

vations lacking objective measures of compliance, which possibly has to be considered a major confounding factor. Also, no data are available on mean doses, which might confound the present findings since, for instance, NET inhibition by venlafaxine only occurs at higher doses (225 and 375 mg/day) [35]. Furthermore, none of the patients was drug naïve with respect to antidepressant medication, but no detailed data on the type of antidepressant premedication were available. Antidepressant treatment prior to the present investigation might have influenced the presently evaluated treatment response, which could not be controlled for in detail. In addition, since, a priori, only patients with a treatment cycle of at least 6 weeks from baseline with one particular treatment regime were included in the present study, dropouts due to nonresponse could not be accounted for. Unfortunately, this also implies that there was no information on medication switches that could have helped in accounting for tolerability, which had been suggested to be influenced by 5-HTT gene variation before [7]. Finally, we cannot rule out improvement due to other factors than medication. However, all patients received the same treatment in terms of psychosocial support and general treatment such as exercise.

In conclusion, the present findings do not support an impact of the NET and 5-HTT genes on antidepressant treatment response in major depression. Interestingly, however, there might be an impact of a -/CT ins/del polymorphism in the enhancer domain of the NET gene on treatment response in melancholic depression, which remains to be functionally investigated in future studies. The observed significant influence of 5-HTT gene variation on antidepressant treatment in anxious depression points to anxious depression as a potentially diagnostic entity of its own, requiring specific diagnostic and therapeutic attention.

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