Candidate Gene Polymorphisms in the Serotonergic Pathway: Influence on Depression Symptomatology in an Elderly Population

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Background: Depressed mood is a major concern in the elderly, with consequences for morbidity and mortality. Previous studies have demonstrated that genetic factors in depression and subsyndromal depressive symptoms are no less important in the elderly than during other life stages. Variations in genes included in the serotonin system have been suggested as risk factors for various psychiatric disorders but may also serve as candidates for normal variations in mood.

Methods: This study included 684 elderly Danish twins to investigate the influence of 11 polymorphisms in 7 serotonin system genes on the mean level of depression symptomatology assessed over several years, reflecting individuals’ underlying mood level.

Results: A suggestive association of sequence variations in genes responsible for the synthesis (TPH), recognition (5-HTR2A), and degradation (MAOA) of serotonin with depression symptomatology was found, although the effect was generally restricted to men. We also found that a specific haplotype in VMAT2, the gene encoding the vesicular monoamine transporter, was significantly associated with depression symptoms in men (p = .007).

Conclusions: These results suggest that variations in genes encoding the components of serotonin metabolism may influence the basic mood level and that different genetic factors may apply in men and women.

Key Words: Depression symptomatology, genetics, mood disorders, polymorphisms, serotonin system

Both major depression and subsyndromal depressive symptoms have been associated with late-life morbidity, cognitive decline, functional disability, and mortality (Green et al 2003; Saz and Dewey 2001; Stek et al 2004). Although reported depression prevalence rates among the old vary (Beekman et al 1999; Blazer 2003; Garetti et al 2002), depressive symptoms seem to increase steadily with age, thus signifying that depressed mood in the elderly is a major concern (McGue and Christensen 2003; Takkinen et al 2004).

Several twin studies have indicated that genetic factors and nonfamilial environmental factors are the main contributors to major depression as well as depression symptomatology, with the genetic contribution accounting for no more than 16%–37% (Gatz et al 1992; McGue and Christensen 1997; Sullivan et al 2000). A recent Danish twin study assessing depression symptomatology in elderly Danish twins found, however, that the average level of mood assessed using repeated measures over several years is considerably more heritable (64% in men and 69% in women). This indicates the existence of a general underlying mood level that is influenced by genetic factors and suggests that any deviations from this average level that may be observed in single occasion assessment would consequently be caused mainly by the effect of time-specific environmental effects, such as recent life events (McGue and Christensen 2003). According to this hypothesis, identification of genes associated with this highly heritable underlying mood level may be beneficial for studying the genetics of mood regulation and possibly even facilitate extrapolation to the study of risk factors for depression susceptibility. In a cross-sectional study of Danish twins aged 45 to >95 years, Johnson et al (2002) further demonstrated that the heritability of depression symptomatology does not change with age, implying that genetic factors are no less important in older age than at other life stages.

Several lines of evidence have established that depressive symptoms are associated with functional impairment of the serotonin (5-hydroxytryptamine, 5-HT) system, which is involved in regulation of mood in humans. For example, many antidepressants in current use are directed against receptors and enzymes in this system (Tammenga et al 2002), retardation of serotonin neurotransmission by tryptophan depletion has been shown to precipitate depressive symptoms (Quintin et al 2001; Smith et al 1997), and abnormalities in brain serotonin transporter and receptor function has been found in depressed patients (Bhagwagar et al 2004; Malison et al 1998). Accordingly, the genes involved in the synthesis, transport, and degradation of serotonin have gained much attention in the effort to unravel the underlying genetic basis of depression. In the serotonin transporter, two extensively studied functional sequence variations—a long–short allele polymorphism located in the upstream regulatory region and a variable number of tandem repeats (VNTR) polymorphism in intron 2, both affecting the transcriptional activity (Heils et al 1996; Lovejoy et al 2003)—have been associated with a number of psychiatric diseases, although results have not been consistent (Bellivier et al 1998; Caspi et al 2003; Lotrich and Pollock 2004; Serretti et al 2002). Similarly, candidate polymorphisms in several serotonin receptors have been suggested to be involved in mood disorders (Huang et al 2003, 2004; Jansson et al 2003; Lemonde et al 2003; Strobel et al 2003). Thus, a −1019C/G polymorphism positioned in the serotonin receptor 1A promoter was previously suggested to affect gene expression, consequently leading to changes in serotonergic neurotransmission (Lemonde et al 2003).

In the serotonin transporter, two extensively studied functional sequence variations—a long–short allele polymorphism located in the upstream regulatory region and a variable number of tandem repeats (VNTR) polymorphism in intron 2, both affecting the transcriptional activity (Heils et al 1996; Lovejoy et al 2003)—have been associated with a number of psychiatric diseases, although results have not been consistent (Bellivier et al 1998; Caspi et al 2003; Lotrich and Pollock 2004; Serretti et al 2002). Similarly, candidate polymorphisms in several serotonin receptors have been suggested to be involved in mood disorders (Huang et al 2003, 2004; Jansson et al 2003; Lemonde et al 2003; Strobel et al 2003). Thus, a −1019C/G polymorphism positioned in the serotonin receptor 1A promoter was previously suggested to affect gene expression, consequently leading to changes in serotonergic neurotransmission (Lemonde et al 2003). In the serotonin receptor 2A two coding polymorphisms, a silent...
102C/T and a functional 452H/Y substitution, as well as a promoter region polymorphism, −1438A/G, have been widely investigated (Anguelova et al 2003; Fanous et al 2004; Jansson et al 2003; Minov et al 2001). Again results have been conflicting; however, the 452H/Y polymorphism was recently shown to have a possible effect on signal transduction (Hazelwood and Sanders-Bush 2004), and the −1438 polymorphism has been found to modulate transcriptional activity of serotonin receptor 2A (Parsons et al 2004).

One of the best-known polymorphism in the tryptophan hydroxylase gene encoding the rate-limiting enzyme of serotonin biosynthesis is a 218A/C substitution in intron 7. This polymorphism may not be of functional relevance per se but has nevertheless been associated with numerous mood disorders (Du et al 2001; Serretti et al 2001; Tan et al 2003a). The same situation applies to two polymorphisms in the monoamine oxidase A gene catalyzing the breakdown of serotonin, a VNTR in the promoter region and a CA di-repeat in intron 2 (Eley et al 2003; Preising et al 2000; Schulze et al 2000). Although the promoter VNTR was previously reported to affect the transcriptional activity of transfected cells (Sabol et al 1998) a later study failed to find an association of the VNTR with either expression level or enzyme activity in human brains (Balcuniene et al 2002). The suggested association of these variants with mood disorders may instead be due to linkage disequilibrium with other, as yet unidentified, functional variants.

In this study, we used a systematic approach to investigate the potential association of depression symptomatology with 11 single nucleotide polymorphisms (SNPs) or VNTRs in 7 genes from the serotonin system. The selected genes are implicated in the synthesis (tryptophan hydroxylase [TPH]), the transportation (vesicular monoamine transporter 2 [VMAT2]), serotonin transporter [5-HTT], the recognition (serotonin receptors 2A [5-HTR2A], 1A [5-HTR1A], and 1B [5-HTR1B]), and the degradation (monoamine oxidase A [MAOA]) of serotonin. All but two of the selected polymorphisms (the VMAT2 sequence variants) have previously been associated with mood disorders. The study population was 684 Danish twins from the Longitudinal Study of Ageing Danish Twins, for which depression symptomatology has been assessed up to five times since 1995.

Methods and Materials

Study Population

The participants in this study were drawn from the 1997 survey of the Longitudinal Study of Ageing Danish Twins (LSADT), which includes Danish twins aged 70 years and older, as described in details elsewhere (Skytte et al 2002). In brief, the LSADT is an ongoing longitudinal study initiated in 1995 and repeated every other year with the most recent survey conducted in 2003. Each survey comprises multidimensional face-to-face interviews focusing on health and lifestyle issues, assessment of cognitive and physical abilities, assessment of depression symptomatology and DNA sampling (DNA sampling included from the Danish Scientific-Ethical Committees (file no. VF19960244); the substudy described here was approved subsequently (file no. VF20030011).

Depressive Symptoms Assessment

Depressive symptomatology was assessed using an adaptation of the depression section of the Cambridge Mental Disorders of the Elderly Examination (CAMDEX) as described (McGue and Christensen 1997). The 21 items (16 original CAMDEX items and 5 supplementary items) that directly assessed current depression symptomatology were factor analyzed resulting in two factor scales, the first loading on items reflecting affective content and the second loading on items reflecting somatic complaints (McGue and Christensen 1997). The somatic and affective scales derived from the factor-analytic results were highly correlated (approximately .60), justifying the formation of a total depression score defined as the sum of the two subscale scores. In this study, we used the average total depression score based on one to five assessments for each participant. Of the 684 individuals in the study group, 134 participated in all five assessments (1995–2003), 221 participated in four, 116 in three, 149 in two, and 64 in one.

DNA Analysis

DNA was isolated from full-blood using standard procedures (Miller et al 1988). Genotyping of all SNPs were performed by allelic discrimination using the Taqman technology. Primers and probes were designed using the Primer Express software (Applied Biosystems, Foster City, California). DNA was amplified in a total volume of 10 μL containing 5 μL Taqman Universal Master Mix (Applied Biosystems), 900 nmol/L of each primer (DNA Technology), 50–250 nmol/L of each TAMRA-probe (MWG Biotech, Ebersburg, Germany) or MGB-probe (Applied Biosystems), and ~10 ng template DNA. Primer and probe sequences as well as modifications of the procedures applied to the specific reactions are described later under each polymorphism. Polymerase chain reaction (PCR) was performed in the ABI Prism 7700 using the conditions recommended by the manufacturer (Applied Biosystems) and analyzed using the Sequence Detection System software.

DNA amplification of all VNTRs was performed in a total volume of 6 μL containing 1× PCR buffer, 200 μmol/L of each dNTP (Amersham Biosciences, Piscataway, NJ), 167 nmol/L of each primer (DNA Technology), .18 U Taq DNA polymerase (Sigma, St. Louis, Missouri), and ~10 ng template DNA. The PCR conditions for each VNTR are described subsequently. For detection, all the sense primers were labelled with 6-FAM. The PCR products were resolved on the MegaBACE 1000 according to the manufacturer’s instructions and analyzed using the Fragment Profiler software (Amersham Biosciences).

All SNP genotyping assays included eight replicates of a no template control and two allele specific controls in each run. All VNTR genotyping assays included duplicates of two no template controls and two control samples in each run.

PCR modifications and primers and probes used for each polymorphism were as follows:

TPH: The 218A/C polymorphism (refSNP ID: rs1800532) was amplified and detected using the sense primer 5′-tggtacctcg-agatgaatacatg-3′ and antisense primer 5′-tcatgtgatcatcctccatgc-3′, 200 nmol/L 218A probe 5′FAM-aatagcagctatcacctaa-ggctca-3′, 200 nmol/L 218A probe 5′FAM-aatagcagcatcctcacta-MGB-3′ and 150 nmol/L 218C probe 5′VIC-aatagcag-catcagcactca-MGB-3′.
VMAT2. Two common SNPs, located in intron2 and intron 9, were selected from the NCBI SNP database. SNP1-C/T (rs633399) was amplified using the sense primer 5′-gctcagcgcaggaagt-3′ and antisense primer 5′-tccgttgaaatattctaggt-3′ and detected using 200 nmol/L of the C probe 5′-FAM-ctctgaaattctgtagtgaa-TAMRA-3′ and 200 nmol/L of the T probe 5′-JOE-ctctgaaattctgtagtgaa-TAMRA-3′.

SNP2-C/G (rs4752045) was amplified using the sense primer 5′-ccaggtgcctgccgctgc-3′ and the antisense primer 5′-gtagccagagctctccaa-3′ and detected using 100 nmol/L of the C probe 5′-FAM-ttgtgcgcggctgc-TAMRA-3′ and 200 nmol/L of the G probe 5′-JOE-ttgtgcgcggctgc-TAMRA-3′.

5-HT1B: The 861G/C polymorphism (refSNP ID: rs6296) was amplified using the sense primer 5′-gctcagcgcaggaagt-3′ and antisense primer 5′-tccgttgaaatattctaggt-3′ and detected using 200 nmol/L of the C probe 5′-FAM-ctctgaaattctgtagtgaa-TAMRA-3′ and 200 nmol/L of the T probe 5′-JOE-ctctgaaattctgtagtgaa-TAMRA-3′.

5-HTT VNTR located in intron 2 (IVS2 VNTR) consisting of 9, 10, or 12 copies of a repeat element was amplified using the primers described in Weese-Mayer et al. (2003). The PCR conditions were as follows: denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min; PCR was terminated by extension at 72°C for 7 min and cooled to 4°C. The amplification mix was described earlier with a MgCl2 concentration of 1 mmol/L. The PCR product was amplified and detected using the sense primer 5′-gaatccggatctc-3′ and the antisense primer 5′-ccagcgcagagctctccaa-3′, respectively, were selected from the NCBI SNP database.

5-HT1A: The −1438A/G polymorphism (refSNP ID: rs6311) was amplified and detected using the sense primer 5′-gtatctccatgactgcaacac-3′ and the antisense primer 5′-cgcggacggaacttagtgcttgcttgcaagggacagggagggaggg-3′, 250 nmol/L of the A probe 5′-FAM-aaatgctgcacatcca-MGB-3′ and 200 nmol/L of the G probe 5′-VIC-aagtcggcagactc-MGB-3′. The PCR products varied between 288 and 378 bp. Alleles was divided into a short allele (three copies of the repeat) and a long allele (12 copies of the repeat).

5-HT1B: The 861G/C polymorphism (refSNP ID: rs4752045) was amplified using the sense primer 5′-gctcagcgcaggaagt-3′ and antisense primer 5′-tccgttgaaatattctaggt-3′ and detected using 200 nmol/L of the C probe 5′-FAM-ctctgaaattctgtagtgaa-TAMRA-3′ and 200 nmol/L of the T probe 5′-JOE-ctctgaaattctgtagtgaa-TAMRA-3′.

MAOA: For amplification of the 30 bp VNTR in the MAOA promoter consisting of variants with 3, 3.5, 4, and 5 repeats, the primers described in Jonsson et al. (2003) was used. PCR setup and conditions were as for the intron 2 VNTR in 5-HT1B except that the MgCl2 concentration was 2 mmol/L. The PCR products varied between 288 and 378 bp. Alleles were divided into a short allele (three copies of the repeat) and a long allele (3, 5, 4, or 5 copies of the allele).

The CA di-repeat in intron 2 of the MAOA gene was amplified using the primers described in Furlong et al. (1999). The PCR conditions were as follows: denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 30 sec and extension at 72°C for 30 sec; PCR was terminated by extension at 72°C for 7 min and cooled to 4°C. The amplification mix was as described earlier with a MgCl2 concentration of 1.5 mmol/L; PCR products varied between 108 and 128 bp. Alleles were named consecutively, with allele 1 being the 108 bp product and allele 11 being the 128 bp product.

Statistical Analysis

Depression scores were adjusted for the effects of age and sex before statistical analysis. Specifically, for each wave of data we first log transformed the depression scores and regressed out the effects of age and sex to produce a residual score that was unrelated to age and sex. A mean score was computed by averaging for each individual the number of valid residual scores. To place the resulting mean scores back on the original log scale, a constant of 3.1 was added to all scores.

A previously described logistic regression model was applied to measure the association of genotypes and haplotypes with the depressions symptomatology trait (Tan et al 2003b). We fitted the logit of the genotype or haplotype frequency as a linear function of the trait value for a specific allele or a haplotype, where the slope parameter β measures the genetic association with the trait of interest. The effect of genotypes was assessed using the most frequent homozygous genotype at each locus as reference, and the effect of haplotypes was assessed by setting all other haplotypes at each locus as baseline. Because the MAOA CA repeat is multiallelic this polymorphism was analyzed in the same way as haplotypes to avoid loss of power. Once the parameters are estimated, we can compute the conditional (for the given depression score) or the overall genotype or haplotype frequency using $p(x) = e^\theta(x)/[1 + H(x)]$, where $H(x)$ is the sum of the odds. The odds ratio (OR) or relative risk (RR) of the genotype or haplotype, respectively, for a unit increase in the trait value can be calculated as $e^\theta$.

In haplotype analysis, the parental phases are missing from our data, and thus we used a retrospective likelihood approach to infer the haplotype effects and to estimate the haplotype frequencies (Tan et al 2005). Because our sample contains related participants—that is, twin pairs—significance levels cannot be obtained using the standard errors of our estimated coefficients. To assess the significance, we used bootstrap resampling method to calculate empirical $p$ values as $p = \sum_{i=1}^{B} I(\text{abs}(\hat{\beta}_i) \geq \text{abs}(\hat{\beta}_{\text{obs}}))/B$, where $\hat{\beta}_i$ is estimated from random sample $i$ and $\hat{\beta}_{\text{obs}}$ is from the originally observed data; $B$ is the number of random samples which we set to 1000.

Results

All data are presented for men and women separately because previous studies have pointed out sex differences in genetic influence of depression phenotypes (Zubenko et al 2003). The overall genotype distributions for the investigated polymorphisms are presented in Table 1 along with the tests for genetic association. The table provides the estimated slope and relative

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risk for each genotyped locus using the logistic regression model as described, where the effect of each genotype is assessed by evaluating it against the most frequent homozygous genotype at that locus. Because of the multiallelic nature of the MAOA CA repeat, all genotypes and alleles of this polymorphism are evaluated against the remaining genotypes and alleles at this locus. Using this approach, we found a significant change in several genotype frequencies with increased depression symptom score in men, and an accordingly decreased or increased relative risk, reflecting a decreased depression score in the VMA2 SNP2 C allele carriers (OR = .46, p = .005 for CC and OR = .76, p = .16 for CG individual), an increase in depression score in HTR2A – 1438 AA carriers (OR = 1.97, p = .017), and a decrease in TPH218 heterozygotes (OR = .612, p = .011). Moreover, the results suggest a nonsignificant tendency of association of the MAOA CA repeat allele 8 with a higher (OR = 1.6, p = .095) and HTR1A – 1019 CG heterozygosity (OR = 0.7, p = .092) with a lower depression score.

The results of Table 1 furthermore disclose that a similar pattern was not seen in women, where the only significant association found was between the MAOA 4/8 genotype and a decreased depression score (OR = 0.67, p = .008).

In Table 2, the analogous results for the common haplotypes formed within VMA2, 5-HTT, HTR2A, and MAOA are shown for
men and women. The effect of each haplotype is evaluated against the remaining haplotypes at that locus. From these results, it appears that the decrease in depression symptom score was mainly restricted to male participants, thus indicating that different genetic factors may contribute to levels of depression symptomatology in men and women (Zubenko et al 2003). However, except for the VMAT2 gene, the candidate gene polymorphisms we investigated have all previously been found to be associated with various psychiatric disorders, such as bipolar disorder, unipolar major depression, schizophrenia, and suicidal behavior. Results have, however, been inconsistent, often with initially positive findings followed by lack of support in subsequent replication studies, a fact that may be attributable to the use of small sample sizes. Accordingly, several recent meta-analyses on the impact of the most extensively studied gene in this respect (i.e., 5-HTT) on bipolar and unipolar depressive disorders, concluded that although many studies report little or no effect, the compilation of all studies published do point toward an association of the HTTLPR short allele with increased susceptibility to affective disorder (Angelov et al 2003; Lasky-Su et al 2005; Lotrich and Pollock 2004). Similarly, (Gonda and coworkers 2005) recently showed that the HTTLPR short allele was also associated with physical–vegetative subthreshold depression in younger women when using the Zung Self-Rating Depression Scale.

In apparent contradiction to these results we find no association between serotonin transporter polymorphisms and depressive symptomatology assessed over several years in either men or women. One explanation for this may be that, although reasonably large, our sample size may still hold insufficient power to detect a weak contribution of this gene variation to a complex phenotype such as depression symptomatology. Another explanation may be that changes in the expression of 5-HTT are not associated with each individual’s basic level of mood per se but rather exert an effect on the susceptibility to deviate from this underlying level as a response to life circumstances, a hypothesis that is in agreement with the large prospective longitudinal study conducted by Caspi et al (2003) indicating that carriers of the short allele exhibited increased vulnerability to depression in relation to stressful life events. Furthermore, Nakamura et al (2000) suggested that the genetic influence of

| Table 2. Association Between Haplotypes and Adjusted Depression Symptom Score |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Frequency (%)   | Slope (β)       | Empirical p Value |
| **Men (n = 230)**              |                 |                 |                 |
| VMAT2:  C_1/C_2               | 11.4            | −0.032          | 0.955           |
| SNP1 C/T:  C_1/G_2           | 14.2            | 0.371           | 1.448           |
| SNP2 C/G:  T_1/C_2           | 37.0            | −0.584          | 0.558           |
| 5-HTT:  S_1/S_2              | 12.3            | −0.496          | 0.609           |
| IVS2 VNTR s/l:  L_1/L_2      | 32.8            | 0.105           | 1.111           |
| HTR2A:  A_1/C_2              | 26.7            | 0.133           | 1.142           |
| 452 C/T:  G_1/C_2            | 66.2            | −0.170          | 0.844           |
| MAOA:  S_1/S_2               | 6.5             | −0.148          | 0.862           |
| 30 bp VNTR s/l:  S_1/S_2     | 14.8            | 0.505           | 1.657           |
| CA repeat:  S_1/10_2         | 6.5             | −0.599          | 0.550           |
| **Women (n = 454)**          |                 |                 |                 |
| VMAT2:  C_1/C_2               | 9.4             | 0.047           | 1.048           |
| SNP1 C/T:  C_1/G_2           | 18.3            | −0.141          | 0.686           |
| SNP2 C/G:  T_1/C_2           | 27.8            | −0.002          | 0.998           |
| 5-HTT:  S_1/S_2              | 44.5            | 0.066           | 1.068           |
| IVS2 VNTR s/l:  L_1/L_2      | 37.5            | −0.098          | 0.907           |
| HTR2A:  A_1/C_2              | 31.4            | 0.003           | 1.003           |
| 452 C/T:  G_1/C_2            | 2.2             | 0.110           | 1.116           |
| MAOA:  S_1/S_2               | 33.1            | 0.024           | 0.976           |
| 30 bp VNTR s/l:  S_1/S_2     | 59.2            | 0.063           | 1.065           |
| CA repeat:  S_1/10_2         | 3.1             | 0.386           | 0.680           |
|                                |                 |                 |                 |

The slope and relative risk (RR) are calculated using all other haplotypes at the locus as reference.

The estimated overall haplotype frequencies in men and women, respectively.

The relative risk of the haplotype for a 1-unit increase in the depression score.

Only haplotypes with a frequency higher than 5% are included.

Discussion

In this study, we evaluated the influence of 11 polymorphisms located in 7 genes involved in the serotonin system on mean level of depression symptomatology assessed multiple times, used as a reflection of an individual’s underlying mood level. By applying a logistic regression model developed to infer an effect of specific genotypes or haplotypes on nonnormally distributed quantitative traits, we found significant associations of depression symptoms score with genetic variations in several of the genes involved in serotonin metabolism. Interestingly, the effect was mainly restricted to male participants, thus indicating that different genetic factors may contribute to levels of depression symptomatology in men and women (Zubenko et al 2003).
5-HTT may be more complex than generally assumed. They reported finding additional alleles within the 5-HTTHTTLPR, suggesting that the short and long allele should be divided into different subtypes that may be associated with a modification of the presumed functional consequence of the short and long allele on transcriptional activity (Nakamura et al 2000; Sakai et al 2002).

The meta-analysis reported by Anguelova et al (2005) also included the serotonin receptor HTR2A. They found no evidence that the HTR2A 102T/C polymorphism, which is in almost complete linkage disequilibrium with HTR2A – 1438, was related to bipolar or unipolar depression (Anguelova et al 2003). In agreement with our results, however, a large study examining 1511 elderly Swedish twins demonstrated an association between the HTR2A – 1438A allele and depressed mood using the Center for Epidemiologic Studies Depression Scale (CESD) as their measure of depression (Jansson et al 2003). It is interesting to note that the association was only evident in male subjects, consistent with our findings.

In addition to the association of the HTR2A – 1438 polymorphism with depression symptomatology, we also found evidence suggestive of an association to polymorphisms in the genes responsible for the synthesis (TPH), transport into synaptic vesicles (VMAT2), and degradation of serotonin (MAOA). Considering the role of these genes in the regulation of serotonin availability at the synaptic cleft, it makes sense that sequence variants in TPH, MAOA, and VMAT2 may have an influence on the underlying mood level. Furthermore, TPH and MAOA was previously reported to be associated with both unipolar and bipolar disorder, although results are again conflicting (Furlong et al 1999; Preisig et al 2005; Serretti et al 2001). Interestingly, Serretti et al (2001) found that even though the TPH 218A/C polymorphisms were not associated with major psychoses overall, patients being TPH CC homozygotes scored higher than A allele carriers, when assessing depression symptomatology. Slightly contradicting this finding our results actually point toward the 218AA homozygotes scoring higher than the heterozygotes, although CC homozygotes still had the highest mean depression symptom score. Although this may seem counterintuitive, it could be an example of the phenomenon of molecular heterosis, defined as the situation in which subjects heterozygous for polymorphisms display a lesser or greater effect on the trait than both homozygotic groups. One explanation for this phenomenon proposes that this may follow from interaction with another, genetic or nongenetic, risk factor that causes a hidden stratification of the study population (Comings and MacMurray 2000). Molecular heterosis has previous been reported for polymorphisms in TPH as well as in other genes putatively involved in psychiatric diseases (Comings et al 1999; Feusner et al 2001; Reuter and Hennig 2005), thus emphasizing that this phenomenon may be of importance when conducting association studies concerning the genetics of affective disorders.

Our results could not confirm the previously reported association between the MAOA promoter VNTR and depressive disorders or neuroticism (Du et al 2002; Eley et al 2003; Schulze et al 2000). We did find, however, a suggestive association between some MAOA CA repeat alleles and depression symptomatology, indicating that genetic variations in MAOA may affect underlying mood to some degree, perhaps through an influence on MAO expression level or activity. This hypothesis is in agreement with Balciuniene et al (2002) who found an association of MAOA enzyme level in human brains with a particular three polymorphism based MAOA haplotype, even though the promoter VNTR did not associate independently (Balciuniene et al 2002) and with a recent report from Jansson et al (2005) demonstrating that MAOA haplotypes, based on four SNPs, were correlated with platelet MAO activity. The latter, however, failed to find a direct association of the established haplotypes to depressive symptomatology as assessed using the CESD scale (Jansson et al 2005), suggesting that only a subgroup of individuals with these particular haplotypes carry a functional MAOA polymorphism.

The synaptic vesicular amine transporter VMAT2 is required for transportation and exocytotic release of serotonin into the synapses and thus plays a critical role in neurotransmission. Nevertheless, studies on the relevance of genetic variations in the VMAT2 gene are scarce, and this is the first report of a genetic association between VMAT2 and depressive symptoms. To our knowledge, at the time this study was initiated, no common polymorphisms had previously been investigated in VMAT2, and hence the two polymorphisms examined in this study were arbitrarily chosen among available common SNPs. Recently, however, Lin et al (2005) screened the promoter region of VMAT2 for sequence variations and found several putative functional polymorphisms that may affect promoter activity and furthermore identified a haplotype that was associated to alcoholism (Lin et al 2005). Another recent article also reported an association between VMAT2 sequence variations and alcohol dependence, especially in men (Schwab et al 2005). These results and our own clearly suggest that this gene should be included when considering candidate gene polymorphisms in psychiatric disorders. Accordingly, we plan to extend our study of VMAT2 gene variations to include the haplotype-defining SNPs described.

Of note, because the VMAT2SNPs were arbitrarily chosen and judged by the position of the considered polymorphisms in VMAT2 as well as in TPH and MAOA, there is no obvious reason to believe that any of these are causatively related to the expression of depressive symptoms. It is more likely that they are in linkage disequilibrium with nearby positioned, and as yet unknown, sequence variations within the genes or in regulatory regions that exert the actual influence on depression symptomatology.

The main limitation of this study is the concern of false-positive results because of multiple testing. Correcting for multiple testing using the false discovery rate, as suggested by Benjamini et al (2001) thus left only the VMAT2 T_C2 association significant. Although not as strict as a Bonferroni correction, we believe that the false discovery rate method is still conservative, given that all polymorphisms investigated in this study, except the VMAT2 SNPs, have previously been associated with various affective disorders.

A major strength of this study is that the samples used for the association study are drawn from the same LSADT twin population in which a high heritability of depressive symptomatology was originally shown, applying the same diagnostic tool and phenotype definitions. Furthermore, because of the limited immigration in the older cohorts in Denmark and the high participation rate in the LSADT study, the twin population used is genetically homogenous and not vulnerable to population stratification, which makes this sample well suited for studying the influence of genetic factors in depression as well as other traits. Accordingly, this elderly twin population has previously been used for the investigation of the impact of several genetic variations on longevity and various age-related phenotypes (Bathum et al 2001, 2004; Bladhbjerg et al 2006; Christiansen et al 2004a, 2004b). One notable application of twin studies is the possibility of estimating the contribution of each polymorphism with a recent report from Jansson et al (2005) demonstrating that MAOA haplotypes, based on four SNPs, were correlated with platelet MAO activity. The latter, however, failed to find a direct association of the established haplotypes to depressive symptomatology as assessed using the CESD scale (Jansson et al 2005), suggesting that only a subgroup of individuals with these particular haplotypes carry a functional MAOA polymorphism.
investigated to the overall heritability. The magnitude and gender-specific nature of the associations found in this study do not justify such calculations, however, because this requires a large number of twins.

Finally, it should be noted that although often used for interviews with depressed patients, the CAMDEX depressive symptom score applied in this study is not suited for classification into clinical depression of varying severity but rather represents a reflection of the normal variation in mood. Thus, we do not know the implication of the depression symptomatology score in relation to clinician-diagnosed depression. Although this study does not specifically add evidence to a relationship between a high depressive symptom score and liability to clinical depression, we do find that a number of the genes that have generally been associated with risk of depression and other psychiatric diseases also appear to influence expression of depression symptoms. As could be expected for a complex phenotype such as mood, the contribution of each of the disclosed associations is small, suggesting that a variety of sequence variations within genes involved in regulation of mood may contribute to the underlying mood level of each individual.

In conclusion, our data suggest that sequence variations in genes involved in serotonin metabolism not only may confer small contributions to susceptibility for various mood disorders but also may influence the normal variation in each individual’s underlying mood.

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